THE RETICULO-ENDOTHELIAL SYSTEM IN GROWTH AND TUMOUR FORMATION

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PREFACE

THE investigations described herein were carried out in the Royal Marsden (Cancer) Hospital and in the Anatomical Department of the Royal College of Surgeons of England while holding a Gordon Jacob Research Fellowship at the Hospital and a Prophit Research Scholarship at the College I should like to thank the Trustees of these Funds for their financial aid Obtaining and staining the human material proved extremely laborious and entailed much waiting in operating theatres Owing to the nature of the staining methods used and the difficulty of fixation of the stained material the experiments often had to be repeated many times before proving successful

I should like to thank all the consultant surgical staff of the Royal Marsden Hospital for their co-operation and forbearance in making material available to me Sir Clement Price Thomas Sir Russell Brock Mr W P Cleland F R C S and Mr O S Tubbs of the Brompton Hospital Mr W P Winterton F R C S of the Middlesex Hospital Mr D N Mathews F R C S of the Hospital for Sick Children Great Ormond Street Mr Terence Ward F R C S of the Queen Victoria Hospital East Grinstead Mr C W F Burnet F R C S Surgeon to the West Middlesex and Hounslow Hospitals Miss A H Baker F R C S Mr E O Harris F R C S Mr C P Malley F R C S Surgeons to Hounslow Hospital all of whom kindly supplied or allowed access to histological material

The histological work on human material was carried out personally in the laboratories of the Royal Marsden Hospital and I am greatly indebted to Dr J W Whittick the former Director of the Pathological Laboratory and to Dr N F C Gowing his successor for accommodating me and for much help and advice The chief technician Mr G C Chadwin has come to my aid over many technical problems The animal work was done either in the anatomical laboratories of the Royal College of Surgeons or in the Institute for Cancer Research At the former Mr J Edwards chief technician gave me great help

Some of the photographs were taken in the Photographic Department of the Institute of Cancer Research by Mr Frank Speed and Mr K G Moreman to whose skill I am much indebted Others were taken personally and the technical difficulties met with in photographing thick sections and unequally stained material as mentioned in the text account for their poor quality To Mr J H Wood M P S and Miss M E Brighton M P S of the Pharmaceutical Department of the Royal Marsden Hospital I should like to record my sincere thanks for preparing endless sterile solutions of dyes for vital injections

To Dr P E T Hancock F R C P Senior Physician to the Hospital I should like to express my sincere gratitude for constant support and encouragement during this work and to Professor A Haddow Director of the Institute of Cancer Research for help on various occasions and for kindly reading the manuscript To Professor

Gilbert Causey of the Royal College of Surgeons who has latterly been guide and mentor I cannot express my indebtedness He read the original manuscript and made much trenchant and constructive criticisms which I have done my best to meet

I should like to thank very sincerely my former secretary Miss G E Woodgate for endless work and help involved in writing this book and other papers and Mr George Deed of Messrs Henry Kimpton for his kind help in all matters connected with publishing

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PART I

INTRODUCTION AND EXPERIMENTAL OBSERVATIONS

CHAPTER I

THE RETICULO ENDOTHELIAL CELLS AND HISTIOCYTIC SYSTEM

In 1863 v Recklinghausen described in the inflamed cornea and in the omentum of different animals amœboid cells which could be distinguished from pus corpuscles Twenty years later Metchnikoff called attention to the fact that in addition to the free mononuclear phagocytic cells of the blood and the lymph fluid there were present in the connective tissue and in certain organs sessile cells which in spite of their being fixed were able to throw out amœboid processes and to seize minute bodies such as foreign blood cells He found these cells in the liver spleen lymph nodes and central nervous system Emphasizing their phagocytic properties Metchnikoff (1905) grouped the free and fixed large mononuclear cells together as macrophages and distinguished them from the microphages the leucocytes of the circulating blood Shortly after Metchnikoff s first reports Wyssokowitsch (1886) published his ob ervations on the freeing of the blood stream from injected micro organisms by the phagocytic activity of the cells lining certain blood vessels Marchand (1897) came to the conclusion that the amœboid phagocytes of the loo e connective tissue were not transformed emigrated lymphocytes but were formed locally from certain connective tissue cells which were arranged especially about the small blood vessels and which he called adventitial cells

A considerable help in differentiating the cells of the normal and inflamed connective tissue and in tracing their origin was offered by the introduction of the acid vital stains Ribbert (1904) was the first to make successful use of the new method by injecting rabbits with lithium carmine He found the dye granules in *certain liver cells in the epithelial cells of the renal tubules in the endothelial cells* of the liver spleen bone marrow and adrenal in the sinus endothelium of the lymph nodes in the reticular cells of the thymus and in the cells of the splenic pulp Ribbert also observed that the same cells were able to take up from the blood other substances such as iron and fat The great progress in vital staining with relatively non toxic acid colloidal dyes was due to Ehrlich s studies on chemical *constitution and cellular affinity Bouffard (1906) found Ehrlich s benzidine dyes* (trypan blue and trypan red) and triphenyl methane dyes (pyrrhol blue and isamine blue) particularly suitable for vital staining and it was with these dyes e pecially pyrrhol blue that Goldmann (1909 1911 1912) carried out his experiments on the significance of vital staining and demonstrated the great affinity of Metchnikoff s macrophages Ranvier s (1890) clasmatocytes etc for acid vital stains These

1925) A medium dispersion appears to be best Very finely dispersed dyes like Congo red pass out of the cells quickly and are stored very little or not at all Coarsely dispersed substances precipitate very easily especially when given sub cutaneously or intra peritoneally and the locally formed deposit restricts the vital staining to the cells in the vicinity Lithium carmine trypan blue and most of the other acid vital stains are sufficiently stable not to precipitate in the body fluids Adsorbed by the phospholipid protein complex of the plasma the dyes seem to stain plasma diffusely

Not only vital dyes however but many other substances and colloids may collect in the reticulo endothelial cells of living animals These include the carbon particles of lamp black graphite Indian ink many metal hydrosols such as saccharated oxide of iron colloidal silver bismuth gold carmine and quartz particles Hæmo globin behaves like acid dyes In addition to these colloids red blood corpuscles poly morphonuclear leucocytes (Whitby and Britton 1950) and organisms including viruses bacteria trypanosomes spirochaetes malarial parasites etc also collect in the reticulo endothelial cells (see Jaffé Burrows 1932 Franceschini) However not only foreign substances and particles but also *physiological* substances are concentrated in the reticulo endothelial cells Thus naturally occurring pigments and antibodies collect in them The cells show a great affinity for cholesterol and cholesterol esters (Landau and McNee 1914) Cholesterol administered intra venously is stored throughout the system including the alveolar macrophages Injected lecithin is likewise taken up (Tomkins 1943 McKibbin *et al* 1945) Fat emulsions given intravenously are similarly concentrated in the macrophages (Dunham and Brunschwig 1944 McKibbin *et al* 1945) as are colloidal aggregates of albumin and globulin (Biozzi *et al* 1957) Adrenaline and choline are also concentra ted The Kupffer cells of the liver avidly take up vitamins including vitamins A and D (Drummond and MacWalter 1934) vitamin B (Verzar and Laszt 1936) vitamin B (Giannico 1950) vitamins C and A (Chevremont 1943) vitamins C and D etc (For a review see Franceschini 1955) In the diseases known as lipidoses various abnormal substances such as cerebrosides phosphatides or cholesterol collect in the reticulo endothelial cells Again in diabetes lipids may be stored in these cells It appears therefore that almost all colloidal suspensions dyes lipids sterols vitamins antibodies pigments adrenaline choline viruses cells such as bacteria spirochaetes red blood corpuscles polymorph leucocytes trypanosomes etc in fact *anything introduced into the body fluids collects and is concentrated in the reticulo endothelial cells* Electro positive colloids and even crystalloids which are not stored *per se* can also be made reticulo endothelial trop when they are adsorbed by an electro negative phase (v Jancso 1931) such as the phospholipid protein complexes of the plasma

The fixed elements of the reticulo endothelial system and the macrophages are cells of varying shape 15–18 μ in size containing vacuoles and often numerous coarse granules consisting of lipids especially lecithin (see for example Franceschini) These granules are oxyphil and argentophil in staining and consist of doubly refracting material They appear to contain reducing agents No glycogen is present The littoral macrophages in the supporting tissue of the adrenal cortex

stains differentiated the histiocytic wandering cells from the emigrated blood cells better than any other method was able to do The dye granules were also demonstrable in the cells of the taches laiteuses the adventitial cells of the omentum the Kupffer cells of the liver and the reticular cells and endothelial cells of the blood forming organs In inflammation the vitally stained cells became free and developed into amoeboid polyblasts ' The same cells were found to be concerned with cholesterol metabolism (Landau and McNee 1914) In 1914 Kiyono s book on the vital storage of carmine was published and presented the first fairly detailed account of the distribution of vitally staining cells in the body Kiyono gave the name histiocytes (tissue cells) to the amoeboid cells of the loose connective tissue which were endowed with a great affinity for the carmine Aschoff (1924) introduced the collective term reticulo endothelial system for the vitally staining cells Further descriptions of the reticulo endothelial system have been given by Aschoff (1924) Maximov (1927) Cappell (1929) Du Bois (1934) Jaffe (1938) Chevremont (1948) Thomas (1949) and Franceschini (1955)

The essential feature of this system of cells is that they take up and concentrate certain dyes and particles injected into the living animal Vital staining with colloidal dyes is the intracellular granular storage of ultramicroscopic particles of these dyes The cells of the reticulo endothelial system consist of fixed or littoral cells found on the reticulin connective tissue fibres forming the framework of various parenchymatous organs the hæmopoietic organs and the pineal pituitary spleen adrenal cortex kidney etc Similar cells known as resting wandering cells histiocytes or macrophages are found in the tissues generally and in the blood They form another part of the reticulo endothelial system In the liver the phagocytic cells are known as Kupffer cells and lie on the reticulin framework of the organ In the central nervous system the cells are seen as the microglia Reticulo endothelial cells also stain by silver methods Thus microglia is stained by silver carbonate impregnation (del Rio Hortega 1927) and also the macrophage cells in the spleen the adventitia of blood vessels (Hortega and de Asua 1921 1924) cardiac and voluntary muscle (Visentini 1931) lymph nodes those lining alveolar walls etc (see Marshall 1956) This may lead to such cells being confused with nervous elements which also stain by such methods

A distinction is usually made between basic and acid vital stains Acid that is electro negative dyes introduced into the circulation are stored chiefly by the reticulo endothelial cells In such conditions they do not enter the brain except in certain areas but permeate the cornea into the aqueous humour and C S F They colour the blood vessels of the iris choroid and pia mater but leave the ciliary and choroidal epithelia unstained When injected into the circulation basic electro positive dyes are adsorbed on to the electro negative colloids (phospholipid protein complexes) of the plasma and so become negatively charged and thus tend to produce similar staining to that of acid dyes Even so they are not taken up to the same extent by the reticulo endothelial cells and tend to pass into the parenchymatous cells for example liver pancreas and brain

In addition to the electro polarity there is another physical property which determines the staining power of a given dye namely the dispersion (v Möllendorff

syncytium It may well be that processes of neighbouring cells may come into sufficiently close relationship with one another that they are only divided by the surface membranes covering each cytoplasm Activity at one cell surface will thus spread to affect the surfaces of other cells In the case of the syncytium of the reticulo endothelial system there is evidence in favour of the latter conception in that individual cells may leave it round off and migrate to other parts

The Reticulo endothelial Cells and Immune Bodies

Immune bodies are concentrated in the reticulo endothelial cells like other substances (Jaffé Franceschini 1955 Biozzi *et al* 1957) It seems probable that they are formed in and liberated from other cells and then pass to the macro phages

Reticulin Fibres

For descriptions of these structures reference may be made to the works of Cowdry (1950) Schafer (1949) Maximow and Bloom (1952) and to the recent review by Wasserman (1956) of our present knowledge of the intercellular components of connective tissue

Reticulin fibres (Gitterfasern) are found in all organs and tissues They are specifically stained by silver salts (silver oxide or carbonate) and hence are called argyrophil fibres Usually they give the periodic acid Schiff (P A S) reaction They run irregularly in all directions They are of different thicknesses the thicker consisting of bundles of the thinner Reticulin fibres are the embryonic form of connective tissue fibres not only in the embryo but wherever connective tissue is developing Both reticulin and collagen fibres are made up of smaller units or microfibrils Argyrophilia probably depends on change in the cement substances between the fibres They are everywhere found in association with the phagocytic cells belonging to the reticulo endothelial system though often large stretches of reticulin fibres are not provided with any cells at all Reticulin fibres supply a yield ing matrix for cells holding them in place (Cajal 1933) Fine networks of such fibres run between and are continuous with collagenous fibres in common collagenous connective tissue The reticulin fibres are especially prominent in the neighbourhood of blood vessels where they are related to the pericytes (macrophages) The net work of reticulin fibres forms a thin membranous sheath around the capillaries and separates them from the other elements of the tissues (Maximow and Bloom) Larger blood vessels as in liver and spleen are supported by reticulin fibres The latter play an important role in the maintenance of the patency of the smaller lymphatics They appear as a net like basket around each fat cell In many organs they form diffuse networks which surround and support the various elements located in their meshes The fibres occur as the framework of lymph nodes thymus spleen and bone marrow In these blood forming tissues a syncytium of reticulo-endothelial cells is spread out on the fibres which enclose the blood cell precursors In the liver the reticulin network supports the liver parenchyma and on it lies the syncytium of Kupffer cells which form a complete wall for the sinusoids and separate the liver cells from the blood In the periportal connective tissue reticulin fibres continue

and pituitary gland appear full of granules The presence of both granules and vacuoles in macrophages however depends on the method of fixation Often the granules are lysed and then the whole of the cytoplasm may be oxyphil in staining while at other times the macrophages contain no granules and the cytoplasm does not stain with eosin but gives a clear appearance for example the monocytes of the blood The cytoplasm appears to contain substances which readily take up or lose water during fixation This may give a shrunken appearance or watery vacuoles may be seen in the cells When particles are ingested they become surrounded by a vacuole The vacuoles are acid in reaction as shown by neutral red Increased phagocytic activity of the macrophages is associated with increased basophilia (see Jaffé)

All investigators of the reticulo endothelial system of cells have remarked on the fact that the *phagocytic properties of the cells of the reticulo endothelial system vary* (see Cappell 1929 Burrows 1932) *Macrophages which are rich in lipid granules do not take up vital dyes* such as trypan blue (see Kusnetzowski 1923 Bullif 1948) *After being taken up by the reticulo endothelial cells circulating materials can often be seen to be transferred to parenchymatous or epithelial cells* carmine for example is passed from the Kupffer cells into the liver cells (Schittenhelm and Erhardt 1925)

In all the work on the reticulo endothelial cells their function has been regarded as purely defensive in character straining off *from the circulation* noxious colloid particles bacteria and protozoa which have entered the body and concentrating anti bodies In the present work an attempt will be made to show that this is but one aspect of the functioning of the reticulo endothelial system

The Reticulo Endothelial System in Lower Forms

Cells with the functional qualities of macrophages occur throughout the verte brates but considerable differences exist in their morphology and distribution A similar system of cells is found in invertebrates above and including the molluscs (see Goldner 1929) They are known as nephrophages because they are thought to *be concerned with cellular excretion In insects the system is composed of the* so called pericardial cells the fat body the haemocytes and the nephrocytes (for a full account see Wigglesworth 1947) In insects pigments carotinoids chlorophyll egg white injected litmus and urates collect in these cells They become filled with albuminoid material at the time of metamorphosis The cells are concerned with the excretion and storage of waste and foreign substances urate particles and dead and dying material

The Significance of the term Syncytium

Reticulo endothelial cells lying on a reticulin framework tend to spread out on this and the processes of neighbouring cells appear continuous Frequent use has been made of the term syncytium in speaking of such a collection of the reticulo endothelial cells of a tissue This term is here used to denote a collection of cells in such close relationship to one another that their cytoplasm is *functionally* continuous so that a stimulus or influence acting on one cell will affect all the cells of the syncytium It is not necessary to postulate cytoplasmic continuity in such a

to Suranyi (1928) injected lipids depress and cholesterol increases the phagocytic activity of macrophages while macrophages which contain large amounts of lipids are non phagocytic Anything which causes local stimulation irritation or inflammation of a part causes dye to collect in the macrophages of an area which normally would not stain or causes increased depth of staining in an area which normally takes up the dye (see Burrows 1932) Such effects may be caused by cauterization of the skin liver spleen lymph nodes etc by local pressure on a tissue by the application of chloroform alcohol formalin mustard digitalis diathermy ultra violet light the application of X rays to skin and other tissues by the exposure of a viscus to air by the production of aseptic inflammation by injection of agar gum and kaolin into the tissues or by implanting sterile celloidin foreign bodies into the subcutaneous tissues or muscles They also occur after the application of a hot water bottle to the skin for example of the belly when vital staining also increases in the subjacent coils of the intestines There is a similar marked increase in phagocytic activity and the number of functioning macrophages after the application of choline (Morin and Poggio 1949) or tyrosine (Dierick 1932) This also occurs in areas of inflammation produced by tubercle bacilli or pyogenic organisms whether these arise spontaneously or are produced experimentally It is also seen around the nodules of scabies in animals or in the areas of skin after the application of tar in the induction of malignant tumours (Kreyberg 1927) It is observed also in wounds produced by physical trauma including operation wounds The same factors cause the collection of vital dyes in the cornea in areas of keratitis During wound healing uptake of dye by macrophages occurs in areas of immaturity of tissues or young scar tissue and this is also found in areas of imperfect recovery after operation or injury and in vaccination scars

Not only dyes but also bacteria and pigments collect in stimulated or irritated areas Thus clinically bacteria including the tubercle bacillus treponema and viruses may become localised to areas of trauma of exposure to cold or burns or in vaccination or tattooing scars Tubercle bacilli settle in an injured joint Treponema appear in areas of injection of agar (luetic reaction) *M leprae* become localised in areas irritated by injection of milk peptones or B C G or in skin eruptions due to drugs (Liban Zuckerman and Sagher 1955) Cortisone and A C T H (Menkin 1953) foreign proteins and antigens (Burrows) likewise collect in areas of inflammation The uptake of P^{3} by inflamed tissues as determined by the Geiger Muller counter is also increased as compared with the normal (Tobin and Moore 1942 Das Gupta *et al* 1956)

Injected or ingested toxic substances may increase the storage of vital dyes throughout the body Thus injections of alcohol or pyramidon cause a general increase in vital staining of macrophages in the rabbit Injections of foreign proteins have a similar effect not only locally but throughout the body General infections and antigen antibody reactions or injections of tubercle bacillus toxin (Bruni and Segré 1929) also produce general increased staining of the reticulo endothelial system

In the brain and spinal cord very little staining occurs after intravenous injection of dyes except around a few areas in the region of the hypothalamus etc Any form

directly into the dense network of similar fibres which surround the sinusoids Reticulin fibres surround all the cells of the anterior pituitary parathyroids suprarenal pineal and other epithelial organs forming a delicate basket like supporting investment The uriniferous tubules of the kidney are likewise enclosed by reticulin A similar covering of reticulin fibres is found around muscle fibres including cardiac muscle Reticulin fibres abound in the respiratory portions of the lung and in the epi peri and endo neural tissues In fact the neurilemma of nerve fibres appears to be formed of this substance (Cruikshank and Hill 1933)

Basement Membranes

These structures exist between epithelia the cells of glandular acini and ducts or serous membranes and the connective tissue on which they lie They are distinct *sharp boundary lines of variable thickness and appear to be a condensation of the* intra cellular substance of the connective tissue at the surface of its contact with the epithelium They consist of a dense network of argyrophil reticulin fibres continuing into those of the connective tissue and of a homogeneous ground substance In some cases basement membranes seem to consist of two layers and between the argyrophil reticulin and the epithelium a homogeneous layer can be detected The basement membrane is intimately attached to the basal surfaces of the epithelial cells which are partially enveloped by a basket work of reticulin fibres On the epithelial surfaces the basement membrane at some places (skin cornea) is provided with minute indentations into which fit corresponding short outgrowths of the basal surface of the epithelial cells In other places as in the convoluted uriniferous tubules the inner homogeneous portion of the basement membrane has circular ridges which seem to fit into grooves in the bases of epithelial cells In some cases the epithelial cells may send processes deep into the connective tissue

Lying in close relationship to the basement membranes for example in the gut epithelium are connective tissue cells which as will be shown later appear to be macrophages In the salivary lacrimal sweat and mammary glands there lie on or in the basement membrane certain cells called myo epithelial cells These also stain vitally with methylene blue and other dyes (see below) It will also be shown that in muscle tissue of all kinds there are found numerous phagocytic reticulo endothelial cells lying on the reticulin fibres surrounding the muscle cells while nerve fibres are surrounded by the reticulin of the neurilemma on which lie Schwann cells which are *phagocytic in certain conditions The original simple anatomical relationships between epithelium and connective tissue are altered in the liver* and most endocrine glands such as pituitary suprarenal etc in which the cells of the organ are enclosed in a reticulin framework on which lie the syncytial reticulo endothelial cells

Factors Affecting Vital Staining and Macrophage Activity

As stressed already the vital staining of and the collection of circulating substances in the macrophages of a tissue after their introduction into the circulation is very variable *and depends on physiological and pathological conditions* (For a description of some of the factors affecting this see Burrows 1932) According

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Lying in close relationship to the basement membranes, for example in the *gut epithelium, are connective tissue cells which as will be shown later appear to* be macrophages. In the salivary, lacrimal, sweat and mammary glands there lie on or in the basement membrane certain cells called myo-epithelial cells. These also stain vitally with methylene blue and other dyes (see below). It will also be shown that in muscle tissue of all kinds there are found numerous phagocytic reticulo-endothelial cells lying on the reticulin fibres surrounding the muscle cells, while nerve fibres are surrounded by the reticulin of the neurilemma on which lie Schwann cells which are phagocytic in certain conditions. The original simple anatomical *relationships between epithelium and connective tissue are altered in the liver* and most endocrine glands such as pituitary, suprarenal, etc., in which the cells of the organ are enclosed in a reticulin framework on which lie the syncytial reticulo-endothelial cells.

Factors Affecting Vital Staining and Macrophage Activity

As stressed already, the vital staining of, and the collection of circulating substances in the macrophages of a tissue after their introduction into the circulation is very variable and *depends on physiological and pathological* conditions. *(For a* description of some of the factors affecting this see Burrows 1932.) According

Hormones and macrophage phagocytic activity

The uterine mucosa at certain stages of the œstrus or menstrual cycle shows deep vital staining (Helmy and Nicol 1951) In animals in the early stage of pregnancy vital dyes collect in the nipples and persist there longest (Goldmann 1912) In the lactating mamma there is an extraordinary increase in the number of functioning macrophages as compared with the non lactating gland During pregnancy there is an enormous increase in the phagocytic activity of the macrophages of all layers of the uterus and parametrium and elsewhere in the body They persist for a long time after parturition Haendel and Malet (1929) point out that injection of ovarian extracts is associated with increased phagocytic activity by reticulo endothelial cells generally but especially in the secondary sex organs (Hofbauer 1926 Fluhman 1928 1932 Hooft 1938 Standart 1940) After cessation of sexual life the activity of reticulo endothelial cells in the uterus is diminished in women (Uebermuth 1933) and is practically absent in spayed animals

Nicol and Helmy (1951) and Nicol and Abou Zikry (1953) found that œstradiol benzoate increased the phagocytic activity of the reticulo-endothelial cells of spleen liver lymph nodes and prostate as estimated by the number of cells staining with trypan blue It may well be that the effect of sex hormones on macrophages is secondary to that on the metabolic activity of their target cells

Removal of the thyroid depresses cell metabolism generally and diminishes the phagocytic activity of the reticulo endothelial cells and the storage capacity of Kupffer cells (Ascher 1924 Jaffe 1938)

The innervation of viscera blood vessels glands epithelia etc

The mixed peripheral nerves and posterior and trigeminal nerve roots contain not only various sizes of myelinated but also unmyelinated fibres These afferent unmyelinated fibres together with the preganglionic finely efferent fibres of the autonomic and the grey rami communicantes constitute the C fibres of Gasser and Erlanger (1929)

Unmyelinated plexiform networks with free nerve endings are widely distributed in the body They are seen in the epidermis (Feindel Weddell and Sinclair 1948) in the dermis in loose connective tissue in between muscle fibres on blood vessel walls (Kuntz and Hamilton 1938) and in all viscera (Fulton 1949 Wyburn Mason 1957a) The endings come from unmyelinated posterior root fibres The simplest form of free nerve ending in the connective tissue is represented by nerve fibres which accompanied by Schwann cells break up by repeated divisions into innumerable thin threads running in various directions supplied with varicose thickenings and located between the collagenous fibres In glandular tissue and in the kidney such nerve fibres form plexuses below and in the basement membranes (see Maximov and Bloom 1952) Numerous unmyelinated nerve fibres can also be found in the central nervous system (see Wyburn Mason 1950) In the skin unmyelinated fibres often penetrate the epidermis and end between the cells as varicose threads (Woollard Weddell and Harpman 1940) In the cornea these free unmyelinated nerve endings are the only type found and pain is said to be the only and is certainly the principal

of injury whether touch local trauma or surgical section the application of various irritating chemical substances local pyogenic lesions or softening causes local accumulation of dye in the microglial cells (see Wyburn Mason 1950) Generalized factors such as anoxia CO gas poisoning alcohol intoxication or concussion, cause *a general uptake of dyes by the microglial cells* Injected cocaine also collects in the macrophages of irritated parts of the central nervous system (Burrows)

When applied to tissues many of the agents which increase vital staining cause vasodilatation of capillaries and increased permeability It might therefore be thought that these factors were responsible for the increased collection of substances in the irritated areas but adrenaline applied with mustard oil to the rabbit s eye prevents vasodilatation and inflammatory œdema though intravenous trypan blue is still localized in the treated eye (see Burrows) The same occurs when cocaine is used instead of adrenaline Studying factitious urticaria Hoff (1927) found that injection of a region of skin with adrenaline rendering it pale and an intravenous injection of Congo red followed by tactile irritants to the injected skin caused a *wheal coloured with Congo red in spite of the absence of vasodilatation* Stasis and exudation will also occur in response to capsicum without any change in the calibre of the blood vessels Vasodilatation alone that is arterial hyperæmia in the absence of damage to the capillaries likewise does not lead to increased exudates and collection *of dye in the affected tissues Again passive congestion has no effect on vital staining* (Kusnetzowski 1923) Mere capillary dilatation or increased blood supply is therefore not responsible for the collection of dyes in the stimulated areas In tissue cultures of macrophages the intensity of storage of dye cannot be increased simply by adding more dye (Wallbach 1928) In order to increase the staining the cells have to be stimulated for example by adding foreign serum Thus *the collection of circulating substances in the macrophages of a stimulated or injured area must be due to a stimulation of phagocytic activity of these cells*

The effects of external influences on the phagocytic activity of macrophages may vary with the dose Thus large doses of X rays paralyse phagocytic activity unlike the stimulating effect of small doses This has been formulated as the **Dustin effect or law** (Dustin 1926) This states that in comparable conditions small repeated doses of drugs (and X rays) stimulate the phagocytic powers of the reticulo endothelial cells and large doses inhibit

Macrophage activity in relation to metabolic processes

Increased metabolic activity of an organ for example contraction of a muscle or stimulation of the metabolic activity of the liver also increases the vital staining of macrophages in relation to the active cells (see Jaffé) Fazzari (1949) showed that when thyroid secretion is stimulated there is an increase in the colloid paralleled by that in the functioning macrophages in the stroma Similarly when the activity of the secondary sex organs is stimulated in pregnancy or after œstrogen injections the macrophage phagocytic activity in these organs increases enormously The phagocytic activity of the reticulo endothelial system of an organ in fact parallels its metabolic activity

glands in the walls of the larger blood vessels around capillaries on the end plates of striated muscle (where they lie on the sarcolemma) in between plain muscle fibres in ciliary and iris muscle in loose connective tissue in the mucosa of the frog s mouth and in the glomus caroticum A number of workers have confirmed the presence of a syncytium of cells with the characters of interstitial cells in many tissues and Dogiel (1894) for example described them in between fat cells Both Ramon y Cajal and Boeke have claimed to show that the syncytium of interstitial cells is in syncytial connection with the sheath (Schwann) cells of the nerve plexus

The interstitial cell syncytium appears interposed between the neurofibrillary strands of the postganglionic fibres and effector cells Ramon y Cajal (1894) did not observe a direct connection of the interstitial cell syncytium with the classical nerve elements He put forward a very cautious hypothesis that in the gut they were influenced by the nerve fibres entering the intestinal wall According to Boeke the interstitial cell syncytium forms a periterminal network with an alveolar structure in which the neurofibrils of the postganglionic sympathetic fibres end and which is thought to produce neuro hormones He believed the cells are neural in nature and that neurofibrils emanate from these cells and end in boutons terminaux against the effector cells

The interstitial cell syncytium (like the Schwann cells) persists after denervation (cutting of the postganglionic nerve fibres) Strong evidence has been produced to show that drugs act on the interstitial cells of the gut (see Boeke) The latter stain with methylene blue and silver methods They may show granules and vacuoles Some including Dogiel (1894) Schabadasch (1930 1934) Nonidez (1936 1937 1939 1943) (see Mitchell 1956) regard them as connective tissue elements Bloom thinks they are probably nervous but may be microglial that is reticulo endothelial in nature Other workers have considered them as Schwann cells with which they are claimed to be syncytially continuous Some authors regarded them as small ganglion elements others as simple sympathetic or sensory cells (Coujard 1943 Feyrter 1952 Meyling 1953 Nelemans 1948 Schaefer 1952) Leeuwe (1937) described their developmental origin from embryonic sympathetic plexuses He distinguished them from Schwann cells Though nerve cells elsewhere do not form a syncytium Boeke stressed the fact that they look like nerve cells and has therefore regarded them as neural in nature and claimed they contain neurofibrils but Maximov and Bloom (1952) deny this Boeke felt they are an integral part of the terminal sympathetic formation and not an accessory system as Ramon y Cajal thought They certainly lie in close relationship to unmyelinated nerve endings and the syncytium may well be interposed between the nerve plexus and the effector cells Whatever their nature such cells undoubtedly will be affected by nervous discharge

Rouget cells or pericytes

Around the capillary vessels are found certain cells variously described as Rouget cells or pericytes the nature of which has been in doubt They appear to be macrophages (Best and Taylor 1950) They stain by silver methods which demonstrate numerous processes encircling the vessels but not closely applied to

modality of sensation present (see Tower 1940) All the evidence suggests that such fibres transmit the diffuse compelling and burning type of pain sensation (see Fulton 1949) It seems that in all tissues including epithelia blood vessels and glandular tissue are found free nerve endings of unmyelinated posterior nerve root fibres Such fibres do not degenerate after ablation of the sympathetic Some tissues such as glandular acini epithelia and blood vessel walls also receive sympathetic fibres

In the posterior nerve roots all the 'afferent' unmyelinated fibres of the peripheral nerves whatever their origin are grouped together laterally Division of the lateral part of these roots cuts these unmyelinated fibres and now, on stimulation of the nerve distal to the section no automatic reflexes occur as they do in intact animals (Ranson and Davenport 1931 Clark Hughes and Gasser 1935) After entering the cord the course of most afferent fibres is well known Those conducting pain and temperature are considered to end against cells in the posterior horns from which relay occurs by way of fibres which cross in the central grey matter to ascend on the opposite side of the cord as the spinothalamic tracts Others pass directly into the posterior columns of the cord and ascend to relay in the nuclei of Goll and Burdach The central branches of the unmyelinated C fibres have a different pathway After entering the cord they pass not into the ascending tracts but into Lissauer's intersegmental tract and thus by short relays in relation to the central grey matter up and down the neuraxis ending in the hypothalamus (Kappers Huber and Crosby 1936 Ranson 1939) Thus the C fibres of the peripheral nerves and posterior nerve roots differ in structure course connections and function from other afferent fibres It seems that glands blood vessels viscera etc are supplied with unmyelinated and finely myelinated nerves derived not only from the autonomic but also from the posterior nerve roots and trigeminal nerve

The ending (? or origin) of unmyelinated nerve fibres in relation to their effector cells has been regarded as occurring in different ways Ramon y Cajal demonstrated a thick plexus of unmyelinated nerve fibres in the connective tissue outside epithelia or glandular acini From these bundles some fibres separate off and ramify several times over the basement membrane Mixed with these fibres were fine simple expansions of what he considered to be multipolar nerve cells lying between the acini of glands These branches terminate freely outside and between the secretory cells or rather in the intercellular cement Furthermore around the autonomic ganglion cells and in the meshes of the plexuses in the Auerbach's and Meissner's nerve plexuses of hollow organs and in the cardiac ganglia are seen a large number of triangular stellate cells (Cajal's interstitial or Kölliker's intercalated cells) with ramified varicose expansions penetrating among the bundles of smooth muscle The expansions of these cells often 'anastomose' with one another (Ramon y Cajal 1894 1933) Cajal regarded both the multipolar cells found around glandular acini and the interstitial cells as neural in nature and concerned with the transmission of the nerve impulses to the effector cells

Boeke (1939–40 1949 1951) using silver and vital methylene blue stains also calls attention to the syncytium of anastomosing 'interstitial cells of Cajal' Cells of similar nature he found in between fat cells in the cornea around sebaceous

The Hellen Zellen System of Feyrter

Feyrter (1953) has described a system of cells in various parts of the body which he calls Hellen Zellen (clear cells) or H Z These may occur singly as island cells (Inselzellen) or collections of such cells called insulares Gangorgan and the whole system was named the diffuse endocrine epithelial organs The cells are found lying on the basement membranes of the acini and ducts of glands including the sweat salivary mammary pancreatic prostate the bulbo urethral (Cowpers) para urethral and Bartholins glands (as so called basket cells) and of epithelia such as that of the gut and urinary and genital tracts The H Z are of varying size and may be bud like flask shaped stellate or three cornered band like or net like The cells usually form a syncytium The plasma contains granules or droplets which show as striations in the peripheral parts of the cells They stain with Sudan III with eosin with silver with the Bielschowsky Cros and Gros Schultz methods often giving a striated appearance and with chrome salts The cells shrink in celloidin preparations and in formalin fixed autopsy material the granules are lysed The cell plasma may then stain diffusely with eosin or silver or be completely unstained and clear (leptochrome) The cells often contain much water and watery vacuoles They are hydrophilic and may swell up and press on neighbouring cells or with certain treatments shrink and leave spaces Chromophil and chromophobe cells may lie next one another All these features are identical with those of reticulo endothelial cells while as already deduced basket cells which form part of the H Z system have many of the features of reticulo endothelial cells Feyrter also states that the littoral and wandering cells of the pituitary and parathyroids and certain cells found round the acini of the thyroid gland have the typical characters of Hellen Zellen He also describes the Hellen Zellen as increasing in number in conditions of chronic inflammation Thus the Hellen Zellen appear to have many of the characteristics of reticulo endothelial cells

The prostate is permeated by a rich nervous network in the meshes of which especially well seen in the fœtus and infants are found typical Hellen Zellen either singly or in groups and considered of neurogenic origin In the pancreas argyrophil basket cells or Hellen Zellen are seen lying on the basement membranes of the acini and ducts others are found lying between the acini while still others may be seen on interlobar septa enclosed in a bundle of nerve fibres associated with ganglion cells *and satellite cells Feyrter and his school thus consider Hellen Zellen* are of neural origin with a close relationship to nerve fibres and regard them as endocrine cells forming a system of peripheral endocrine glands However as indicated the Hellen Zellen have many features of reticulo endothelial cells and macrophages Feyrter in fact hints at this but dismisses it The problem of a chemo receptor function of clear cells is also mooted by Feyrter Hellen Zellen accompanying nerve fibres and lying in relation to ganglion cells nerve fibres and their endings and to glandular acini and epithelia correspond in character and situation to the interstitial cells of Cajal while those accompanying nerve bundles correspond to the perineural cells many of which are macrophages The matter will be reconsidered in the next Chapter

them Not all capillaries appear to be supplied with these cells (Best and Taylor 1950) In inflammatory states the cells may round off and migrate like macrophages (Jaffé 1938) which many workers for example Clark and Clark (1925) and Cappell (1929) believe they are

Basket Cells

Around the acini and ducts of the salivary lacrimal and sweat glands breast pancreas and prostate and the suborbital glands of ruminants and the ducts of Wirsung and Santorini of man and lower vertebrates are found certain cells known as basal myo epithelial or basket cells For a full account of these see Zimmermann (1927) and Feyrter (1953) These cells are found within and lying on the basement membrane of the acini and ducts of the glands They are closely applied to the gland cells and those lining the ducts which they tend to encircle They are branching stellate bandlike flattened or spindle shaped They often extend with *their long axis parallel to that of the gland or in a spiral form They form syncytial* anastomoses or networks one with another They contain fibrillary protoplasm and often granules Feyrter (1953) showed that the cell granules stain with chromic salts and silver by Bielschowsky Cros and other methods (see also Muller 1893) They may be striated and look rather like smooth muscle cells The cells also stain with iron hæmatoxylin and with vital methylene blue (Mislawsky 1909) In the salivary gland these cells are stated to take up injected substances (see Zimmermann) pigments and dyes (see Schaffer 1927) for example in diabetes typhoid fever etc The cells round off become mobile or increase in number in inflammation Coupled with the staining characteristics this suggests they may be of macrophage type According to Feyrter (1953) fine nerve fibres penetrate the basement membrane and end *in relation to these cells They appear very early developmentally The* visceral cells of the glomeruli of the kidney and also those of the stellate reticulum of the enamel organ stain similarly and have a similar appearance (Zimmermann) while Feyrter showed that cells with similar characteristics were found in relation to the nerve plexuses in glands such as the prostate or pancreas

Nervous stimulation or pilocarpine is said to cause contraction of the basket cells (Renaut 1894 Sperling and Koppanyi 1947 Ring and Randall 1947) The basket cells have thus been considered as responsible for compressing the glands and expressing their secretion (for accounts see Schaffer 1927 Zimmermann 1927 Cowdry 1928) However macrophages may similarly change shape when stimulated and since basket cells also occur in the perineural tissues it is difficult *to believe they are contractile*

Krause (1865) and Pfluger (1865) both thought the cells were neural ganglion cells interposed by their processes between nerve fibres on the one hand and the gland cells on the other Others have regarded them as epithelial while still others as mesodermal They lie in a position interposed between the blood and epithelial duct or gland cells They are related to nerve fibres Similar cells are found in the nervous network of the glands such as pancreas and prostate *These cells have many of* the features of reticulo endothelial cells

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Langerhans and Clear Cells of the Epidermis

Masson (1926) described cells which he termed "dendritic" cells lying beneath or between the basal layers of the epidermis on the basement membrane and which he considered to be the melanoblasts. These are to be distinguished from the "*cellules claires*" *or clear cells which are again the same as the tactile cells of* Merkel Ranvier. These "clear" cells he thought were connected with the terminal arborization of nerve fibres. From them arise a number of fine branching filaments, which pass up between the palisade cells for surprising distances and form a network about them. In sections of fresh skin the cytoplasm of these cells blackens with ammoniacal silver nitrate unlike the pigment containing cells of the basal layers. In the layers immediately above the germinal or basal layers but also throughout the epidermis are found the cells of Langerhans (Langerhans 1868) which are non pigmented and star shaped with long irregular processes which penetrate the intercellular spaces and follow the intercellular outlines. No traces of them are seen in slides prepared by ordinary methods. They stain with gold and silver reduction methods staining typical of macrophages (see Marshall 1950). The Langerhans and "clear" cells also give the typical staining reactions for Hellen Zellen (Feyrter 1953). They are found not only in the various layers of the epidermis but also in the hair sheaths, the epithelium of the glans penis and of the mucosa of the mouth. Similar cells are also found in the skin of lower forms including reptiles. Their relationship to nerve endings in the skin has been frequently described. For a full account see Schaffer (1927) and numerous articles on the nervous system of the skin by Masson (1926, 1935). Many have regarded the cells as neural in origin. Others *have considered them as lymphoid wandering cells*.

CHAPTER II

EXPERIMENTAL TECHNIQUES

WHILE a great deal of work has been carried out on the reticulo-endothelial system, the exact distribution of the phagocytic cells in the various organs is still not completely known, nor is the relationship, if any, of the phagocytic cells to the nerve fibres. To try to throw light on the problem it was decided to use vital methylene blue staining of tissues. Methylene blue is an especially useful vital dye. In time all living cells including bacteria, single-celled organisms, the cells of a tissue or cells in tissue cultures reduce its N group, resulting in the formation of a colourless leuco-compound. The rate at which this takes place in the vertebrate body varies with different tissues depending on their content of macrophages and their metabolic rate. Methylene blue being an electro-positive (basic dye), when injected directly into a living tissue of course immediately stains all its constituents, but after a while (varying with the tissue) the dye is found to be concentrated in the macrophages, the Schwann and the interstitial cells and reticulin fibres of the tissues, from which the colour gradually fades also. If the tissue is later exposed to air (blued) for fifteen to thirty minutes, the nerve fibres, especially the unmyelinated, regain a blue colour by which time the colour has usually faded from the macrophages. Such vital staining of unmyelinated nerve fibres occurs both in the peripheral tissues and central nervous system. Dead and injured tissues stain diffusely. Methylene blue solutions are relatively innocuous and can be used for injection into patients without ill effects. Weddell, Harpman, Lambley and Young (1940) used this method of direct injection of methylene blue into the tissues of the living animal for the purpose of investigating nerve distribution. The part to be examined was then rapidly removed, cut into slices, blued and fixed. With concentrations of 0.01% of the dye in normal saline left in the tissues for fifteen minutes they obtained the most satisfactory staining of nerve fibres. With concentrations of the dye from 0.005% to 0.03% staining of the nerves was satisfactory throughout, but with increasing concentration the neurilemma sheath cells, reticulin fibres and macrophages became stained.

An investigation of the nerve supply, the interstitial cells and the reticulo-endothelial cells in normal tissue and in malignant tissue and benign tumours was undertaken by direct injection and infiltration of solutions of methylene blue, trypan blue and other vital and supravital dyes into human material either immediately prior to or after removal from the body, or by repeatedly injecting vital dyes into animals. According to the tissue the strength of methylene blue used in the present investigation was kept between 0.02% and 0.08%, depending on the rate of reduction of the dye and its ease of diffusion, that is the looseness of the tissue. The dye solution was made up fresh for each injection. The preparation made by British Drug Houses especially for vital staining was found

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using dyes chemically related to methylene blue such as toluidine blue thionin etc Attempts to counterstain with neutral red and other dyes were abandoned as they obscured the vitally stained structures By this method nerve fibres and endings Schwann cells interstitial cells and also the macrophages can be examined in various tissues At the same time however the dye often condenses on the reticulin fibres and basement membranes on which lie the macrophages All intact epithelial parenchymal and glandular cells and muscle fibres decolourize the dye very rapidly unless damaged Dead tissue stains diffusely and does not decolourize the dye By this method most of the normal tissues of the body were eventually stained satisfactorily

In order to study further the distribution of the macrophages similar infiltrations of fresh tissue were undertaken using vital dyes chiefly trypan blue Janus green B etc Numerous experiments were carried out to determine the appropriate concentration of the dyes necessary It was eventually found that 0 02% trypan blue or Janus green B gave the best results without hyalase Solutions of 0 5% Indian ink were also used The injections were made directly into the tissue and when using trypan blue and Indian ink after a few minutes the material was placed in 10% formal saline for twenty four hours and frozen sections cut and treated as before

Similar injections were made into numerous human benign and malignant tumour tissue Pieces of some of the same growths which had been supravitally injected in an attempt to show nerve fibres were taken and stained for nerve fibres by Holmes method as described in Bolles Lee (1950) as a check on the methylene blue method

In another experiment five mice bearing spontaneous malignant breast tumours (papillary adenocarcinoma) were injected intraperitoneally with 1 ml per 20 G body weight of 1% saline solutions of trypan blue twice weekly for eight injections The mice were killed the day after the last injection and the tissues both normal and malignant fixed in 10% formalin and examined as before

In a further experiment eight mice of the CH_3 strain bearing spontaneous malignant breast tumours were given six twice weekly intraperitoneal injections of 1 ml per 20 G body weight of 1% sterile isamine blue in normal saline (the concentration recommended by Goldmann (1912)) The animals were killed under anæsthesia and their abdomens and thoraces opened and the whole animal placed in 10% formalin solution The various tissues to be examined were cut with the freezing microtome dehydrated in alcohol and cleared in xylol

Experiments have also been carried out with the vital dye pontamine sky blue which has been used by Weinberg and his co workers (for example Weinberg and Greany 1950) for investigating the lymphatic drainage of organs When injected into a tissue and allowed to diffuse the dye stains both the draining lymphatic vessels and nodes An injection of 5 mls of sterile 2% solution of the dye in normal saline was made into the subcutaneous tissues below the angle of the mandible in a patient with metastatic deposits in the cervical lymph nodes from a carcinoma of the floor of the mouth A similar injection was made into the wall of the stomach in a patient with a carcinoma of the stomach with secondary deposits in the portal lymph

most satisfactory When the tissue is loose the blueing of the nerve fibres can be accomplished in the way described above but in tissue containing much fibrous tissue access of oxygen to the interior of the tissues is impossible and it was found that blueing of the nerves does not occur by the method advocated by Weddell *et al* Furthermore in tissues with a very high rate of metabolism such as liver the nerve fibres usually stain only with great difficulty This is also found with epithelia Sometimes both macrophages and nerve fibres become stained at the same time but usually staining in macrophages has disappeared by the time the nerve fibres stain In order to obtain satisfactory staining of the macrophages and nerve fibres of any tissue owing to the variability of staining of macrophages with the physiological state of the tissue it was usually necessary to carry out large numbers of injections a most tedious and time consuming process To try to surmount some of these difficulties hyalase (as advocated by Weddell) was used in the injection fluid or injected into a tissue five to thirty minutes prior to the dye injection to allow diffusion of the dye and access of oxygen to the interior of the tissues This however proved disappointing in staining macrophages as it caused a diffuse staining of the matrix of the connective tissue and its cells and was finally abandoned as altering the normal conditions of uptake of dyestuffs from tissue fluids by many cells Various other methods of blueing the nerve fibres were tried This included the use of H acceptors such as resorcin (0 015%) *p* aminophenol *p* phenylenediamine and pyrocatechol as advocated by Schabadasch (1936) These were found ineffective Eventually hydrogen peroxide sufficient to render the solutions of 1–2 5 vols strength was incorporated in the saline solution of the dye The method finally adopted was to infiltrate the following solution

Methylene blue 0 02%–0 08% } in normal saline
H_2O_2 1–2 5 vols

Even this method did not always result in uniform staining of all the macrophages of a tissue A method was required to stimulate the activity of the macrophages before injection of the dye It seemed that prior injection of foreign protein might be the answer to this problem Egg white thoroughly beaten up and diluted one in two with normal saline was therefore infiltrated into the tissue a few minutes before the dye solution This produced a marked increase in the number of macrophages and nerve fibres staining The technique finally used was as follows

The piece of tissue in about 1–2 cms cubes was obtained immediately after removal from the body With some tissues no egg white was injected With others infiltration with 2–5 ccs of the egg white solution was carried out as evenly as possible This was followed about 2–3 minutes later by the dye solution It is important not to use too much dye solution as this causes a general diffuse staining The dye solution was kept at blood heat until the injection The tissue was then immediately put into a fresh ice cold solution of 8% ammonium molybdate solution for twenty four hours In order to study the distribution of macrophages and nerve fibres in three dimensions thick frozen serial sections (50–100μ) of the whole specimen were cut mounted dehydrated for twenty minutes in dioxane cleared in xylol and mounted in balsam It was found that similar results could be obtained

last form a syncytium and appear interposed between nerve endings and the effector cells While the neural or neural crest origin of the two former types of cell is generally accepted the nature of the interstitial cells is in doubt Some workers believe them to be ganglion cells others to be cells of neural origin others microglial in nature and still others mesodermal elements Cells with similar characters are found between fat cells in the cornea around capillaries in the walls of the larger blood vessels between plain muscle fibres in relation to striped muscle fibres and in the mucous membrane of the frog s mouth and elsewhere

Langerhans and clear cells in the epidermis also stain by silver methods and have been regarded as neural or as wandering cells

The basket cells and Hellen Zellen of glands and epithelia likewise stain with silver methods are reported as taking up circulating substances form a syncytium and like interstitial cells lie in close relationship to basement membranes and nerve endings apparently interposed between the latter and the effector cells They have also been described in relation to peripheral nerves and autonomic plexuses and regarded as neural in origin

Macrophages or reticulo endothelial cells take up circulating substances including methylene blue and their granules also stain by silver methods as do those of interstitial and basket cells and Hellen Zellen

It appeared important to investigate the relationship of the above cells to one another A number of tissues for example the normal breast fat muscle skin glands and nerves were stained by vital methylene blue trypan blue or Indian ink injections Using methylene blue in the breast the interstitial cells stained and were found to form a syncytium around the acini and ducts (Figs 2 3 30) Other cells of various shapes lying between the acinar cells and those lining the ducts and internal to the basement membrane likewise stained with methylene blue (Fig 2) Sometimes these only lay between the bases of the acinar cells All these cells also stained with trypan blue and Indian ink (Figs 1 4) The cells lying between the duct or gland cells correspond in their situation with the myo epithelial basket cells or Hellen Zellen Between fat cells and plain cardiac and striped muscle fibres and in arteriole walls the cells described as identical with interstitial cells and staining with methylene blue also stained with trypan blue (Figs 5 6 8 9 20 23) The interstitial cells around the capillaries which stain with methylene blue likewise stained with trypan blue and appear in fact to correspond to the pericytes or Rouget cells (Figs 6 17 18) The interstitial cells in the enteric plexuses in the submucosa and the interior of the villi and around the acini of glands stain with both methylene blue and trypan blue (Fig 28) Within the epidermis and outer hair root sheaths and in the mucosa of the mouth are also found cells vitally staining both with methylene blue and trypan blue and also by silver methods and they correspond with Langerhans and clear cells (Figs 10 11 12 13) The Schwann cells and satellite cells of ganglion cells and some of the endo perineural cells accompanying the peripheral nerves also stain with vital methylene blue and trypan blue and by silver methods (Fig 7) The interstitial cells Langerhans cells in the epidermis microglial cells macrophages in the alveolar septa of the lungs in the splenic pulp and around malignant growths may all have similar

nodes and also into the body of the uterus in another subject with a carcinoma of the body of the uterus with secondary involvement of the regional lymph nodes After waiting about fifteen minutes for diffusion of the dye the lymph nodes draining the injected organ were removed placed in 10% formalin for twenty four hours and sections cut as before

By this method of direct injection of non toxic substances into living tissue it is possible to study the structures on which either positively or negatively charged particles or ions become concentrated after escape from the circulation and thus their passage into the body cells in the living state Physiological substances in the circulation may well behave similarly Even so the staining in any given tissue using the same technique is variable As already seen this depends on the metabolic activity of the tissue at the time By direct injection into individual tissues far greater numbers of vitally staining cells can be made out in a tissue than by methods involving parenteral injection of substances and their passage into the circulation In the kidney and central nervous system the appearances are totally different from those obtained by parenteral injections

The microscopical appearance of a macrophage depends on a number of conditions It seems to vary with the physiological activity of the cell A macrophage may have a rounded or ovoid configuration or it may be spread out with fine branching processes looking like a ganglion cell (Figs 4 10 12 15 20 36 39 43 44 54 59 63 69 70) At other times it is elongated and appears spindle shaped like a fibroblast (Figs 18 20 21 24 27) especially when compressed by pressure between nerve collagen or muscle fibres The same is observed in the walls of blood vessels They are often elongated when they lie between epithelial cells (Figs 29 31) When they contain fat droplets they may appear vacuolated This can be observed *in cells below the epithelium of the small intestine Finally the appearance* of macrophages varies with the vital dyes used for example the same cells vitally stained with methylene blue show many more processes than those with trypan blue when they often appear round or ovoid (Figs 10 11 12 13)

Macrophages are found especially in three situations

1 *Perivascularly around arteries capillaries and veins*
2 Perineurally and around ganglion cells
3 In the basement membranes of epithelia and glands

From the experimental observations it early became obvious that many cells which are usually regarded as typical fibroblasts for example some of those lying between collagen fibres in collagenous tissue aponeuroses and *tendon readily stained* vitally and this could be increased by stimulation with foreign proteins

The Probable Identity of Interstitial Cells Hellen Zellen Basket Langerhans and Clear Cells with Macrophages

As described above using vital methylene blue and silver staining methods certain cells have been made out lying in relation to ganglion cells to nerve fibres and to their endings in the effector organs The first are called satellite cells the second Schwann cells and endo perineural cells and the last interstitial cells These



Fig 3 Sweat gland an l duct v tally stained with metl yl ne blue sl owing interst t al cells st ming macrophages round a mi an l d ict and fine beaded unmyelinated fil res supplying both (× 00) Compare with F g 4

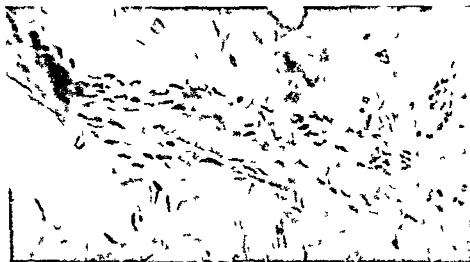


Fig 4 Sweat ducts with macroph ges surround ng the r lls (Trypan blu 00) Com pa w th m thyl ne blue stain g in F g 3 These figures show the ma roj hage nature of the cells a ound the du t



FIG 1 Two a ini of the breast vitally stained with trypan blue showing macrophages tak ing up the dye lying between the glu l cells and in the base ment membrane (× 200) Compare with Fig 2

FIG Two acini of same brea t as F g 1 tally tained with methylene blue also showing ells taking up the dye lying between the gland cells and in the basement memb r ne Beaded nerve fibres with Schwann cells l e close to the latter (× 400) On ompa ing with Fig 1 the essential ma opl age nature of interstit al and basket or myo pithel al ells s ev dent

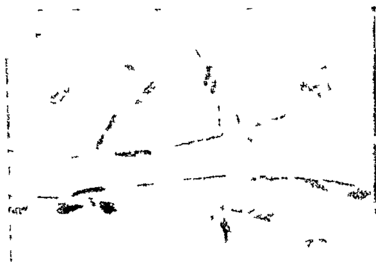


FIG 7 Loose connective tissue supravitally stained with trypan blue showing staining of Schwann cells and indicating their macrophage nature (× 400)

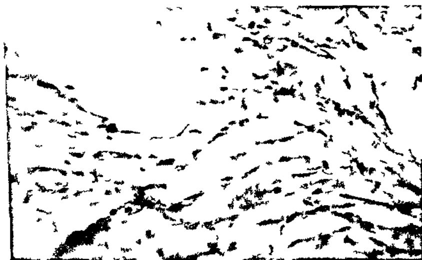


FIG 8 Heart muscle showing macrophage syncytium (myocytes) lying in relation to sarcolemma and continuous with pericytes (Trypan blue × 00) Compare with Fig 3 showing identity of interstitial cells or myocytes with macrophages

FIG Fat with syncytium of interstitial cells of various shapes and beaded unmyelinated nerve fibres lying between the cell (Methylene blue 00) Compare with Fig 6

Capillary rounded by ...

FIG 6 Fat stained with supravital trypan blue showing syncytium of macrophages between fat cells and a branching capillary with pericytes continuous with macrophages between fat cells (× 00) Compare with methylene blue staining (Fig 5) This shows the macrophage nature of the interstitial cells in fat



FIG 11 Epi term Langerhans cells stained witl trypan blue ind ting the r essential ma ropl ge nature Compare w th Fig 10 (× 400)

FIG 1 H ir folli le showing Lang hans cell syncyt um n out hair sh ath st ined w th vit l m thylene blu (× 1 0)

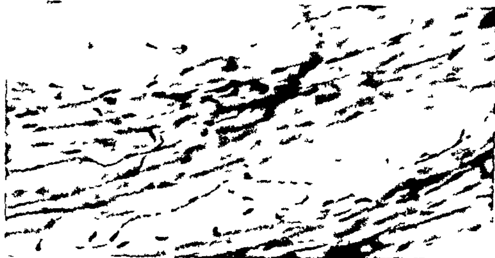


FIG 9 Heart muscle showing macrophages (myocytes) lying in relation to sarcolemma with beaded nerve fibres in relationship to macrophages (Methylene blue × 00)

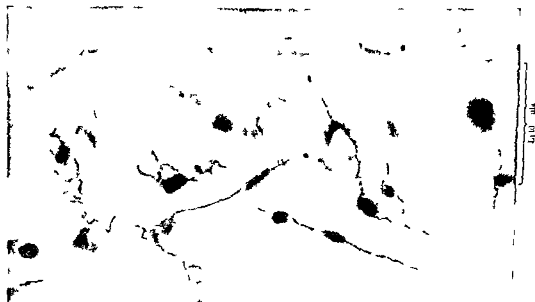


FIG 10 Epidermis Vitally staining cells lying between basal cells and continuous by their processes with Langerhans cells more superficially Below the basal layer the Schwann cells and nerve fibres running up into the epithelium to come into relationship with the processes of the vitally staining cells (Methylene blue 400)

stellate appearances and stain in the same way (Figs 5 10 13 19 24 25 28 29 30 31 32 33 50 53 and 61) It appears likely therefore that *interstitial cells and some at least of the basket (myo epithelial) cells Hellen Zellen clear cells and Langerhans cells are identical in nature and no more than reticulo endothelial cells* and that satellite and Schwann cells are specialized cells of the same nature * Everywhere these cells are found in close relationship to unmyelinated fibres and their endings They would seem to stain temporarily with methylene blue simply because they concentrate it like any other vital dye prior to decolourization

It was mentioned that in these investigations thick sections (50–100 μ) were used in order to be able to follow the meanderings of nerve fibres and their relationship to macrophages Furthermore the processes of macrophages may extend in different photographic planes Because of these facts the technical difficulties of making photographs of the sections have been very great and their quality often poor It has frequently proved impossible to convey on to photographic paper the appearance of the macrophages and nerves and many structures appear out of focus Other workers for example Mitchell (1956) have experienced similar difficulties In studying the microphotographs it is necessary to remember that unmyelinated nerve fibres often have a beaded or dotted appearance due apparently to local varicosities

* It is possible that not all cells found at the sites of basket cells or H Z are macrophage in nature though most of them appear to be so

FIG 13 Hair root showing Langerhans cell syncytium in outer hair sheath but stained with trypan blue (× 200) On comparing with Fig 12 this shows the macrophage nature of the Langerhans cell

FIG 14 Rat spleen supravitally stained with methylene blue showing macrophage syncytium at the circumference of a Malpighian corpuscle Note the absence of staining at the centre of the corpuscle in the right lower corner Compare the appearance of the macrophages with those of vitally staining cells within the epidermis in the basement membranes of epithelia between fat cells in the perichondrium and around the acini of glands in Figs 5 10 13 19 4 25 8 9 30 31 3 33 50 53 and 61 (× 200)

In the autonomic ganglia the multipolar ganglion nerve cells are scattered irregularly throughout the ganglia in groups separated by plexuses of nerve fibres The nerve cells are surrounded by a delicate capsule composed of a connective tissue membrane bordered internally by capsular or satellite cells They are continuous as a syncytium with the Schwann cells accompanying the axons of the ganglion cells The dendrites of the ganglion cells form a network beneath this capsule in relation to the satellite capsular cells and capsular membranes as an intra capsular dendritic plexus The preganglionic fibres entering the ganglion form an inter cellular plexus between the capsules where they may end or pierce the capsules and intertwine with the intracapsular dendrites and the capsular cells as a pericellular plexus (Ranson 1947)

In the enteric plexuses such as Auerbach s and also scattered in the submucosa and interior of the villi and among the muscle bundles are the triangular stellate interstitial cells of Cajal and Kolliker the processes of which interlace to form an irregular network of syncytium

With vital methylene blue injections nerve fibres of all kinds may be stained but the unmyelinated beaded axons seem more easily stained than others Un myelinated fibres within the central nervous system may also stain to the exclusion of other fibres Using methylene blue and trypan blue techniques an intense blue staining is given by bundles of mixed peripheral nerves The axons are found surrounded by very numerous vitally staining epi and peri and endo neural macro phages lying among reticulin fibres and fibroblasts Fine unmyelinated nerve fibres possibly nervi nervorum are also found here

In the loose subcutaneous connective tissue bundles of unmyelinated nerve fibres staining with methylene blue are surrounded by collections of macrophages which appear to be attracted round the unmyelinated fibres (Fig 15) In some sections the perineural macrophages can be shown to diverge from the nerve fibres and to merge imperceptibly with those in the connective tissue generally (Fig 20) Methylene blue in concentrations of 0 03% up to 0 06% trypan blue and Janus green B solutions injected locally are also taken up by Schwann cells (Figs 7 17) In Auerbach s and Meissner s plexuses and in all the layers of the gut the interstitial cells stain vitally with both methylene blue and trypan blue In the loose connective tissue of other organs such as that of the ovary or the senile uterine mucosa the nerve fibres appear to be applied to or come into close relationship with cells which vitally stain with both methylene blue and trypan blue and which thus have the characteristics of macrophages (Fig 16) They almost seem to take the place of Schwann cells

In autonomic ganglia numerous cells staining vitally with either methylene blue or trypan blue can be observed in the connective tissue around or in relation to ganglion cells as satellite cells

Cappell (1929) and others found that peripheral nerve sheaths often stain deeply with vital dyes Ramon y Cajal (1933) and Maximov and Bloom (1932) also state that many of the epi and peri and endo neural cells are macrophages That the neurilemma (Schwann) cells may also stain vitally and phagocytose substances like macrophages has also been reported by Doinikow (1913) Nistri (1949) and others

CHAPTER III*

THE VITAL STAINING AND DISTRIBUTION OF MACROPHAGES AND UNMYELINATED NERVE FIBRES IN NORMAL TISSUES

THE distribution of nerve fibres and macrophages in each of the body tissues will now be considered firstly as revealed by my own findings and secondly from those of other workers. The present knowledge of the distribution of autonomic and unmyelinated nerve fibres to glands, blood vessels, viscera, etc. is largely summarized in the book by Kuntz (1953) from which most of the descriptions of what is known of the innervation of the various tissues are taken.

Macrophages in the Peripheral Nerves and Autonomic System

The ganglion cells of the posterior nerve root ganglia are enveloped by two cellular capsules. The inner is made up of small flat epithelial like satellite or Schwann cells continuous with the Schwann cells enveloping the axons of the peripheral nerves and lying in a thin structureless reticulin membrane which extends along the ganglion cell processes to become continuous with the endoneurium of the nerve fibres. The outer capsule is formed by collagenous fibres and fibroblasts arranged concentrically and containing a dense capillary network accompanied by fine unmyelinated fibres, the nervi nervorum.

The peripheral nerves are enclosed in an epineurium made up of connective tissue cells and reticulin and collagenous fibres mainly longitudinal and fat cells are often found. Each of the smaller fascicules is enclosed in a membrane of concentric connective tissue layers called the perineurium, likewise formed of reticulin and collagen fibres, macrophages and fibroblasts. From this pass fine longitudinally arranged strands of argyrophil reticulin and collagenous fibres and connective tissue cells into the spaces between individual nerve fibres as the endoneurium. The epi-, peri- and endo-neuria are continuous with the leptomeninges. The epi- and peri-neuria contain capillaries and fine unmyelinated nerve fibres, the nervi nervorum. Eventually reaching the periphery where the nerve trunks divide into smaller and smaller branches the connective tissue sheaths become thinner. The smaller branches show no epineurium and the peri- cannot be distinguished from the endoneurium which persists as a reticulin membrane in intimate contact with the neurilemma of the nerve and forms the fibrillar reinforcing sheath (sheath of Retzius or Henle) with associated macrophage cells (Maximov and Bloom 1952). The neurilemmal sheath also consists of reticulin (see Cruikshank and Hill 1953). Both myelinated and unmyelinated fibres are accompanied throughout their course by Schwann cells. The Schwann cells are known to form a syncytium through which axons pass (compare Fig 151—Stohr 1951).

* For those not wishing to read through lengthy accounts of the distribution of macrophages in individual tissues most of this chapter may be omitted and the reader referred to the findings in certain pathological conditions and the summary at the end of this chapter (pp 78–84).

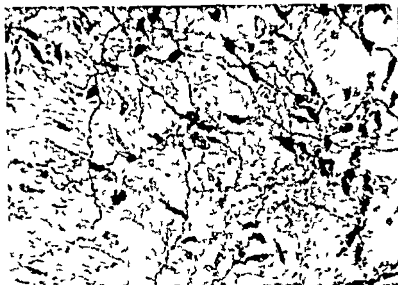


FIG 16 Connective tissue of ovary Nerve fibres passing into close relationship with macrophages (Methylene blue × 00)



FIG 1 Loose connective tissue supravitally stained with trypan blue showing a capillary with staining of pericyte and of neighbouring Schwann cells (00)

these cells and the axons of the preganglionic fibres end in relation to the capsule cells which are thus partly interposed between the preganglionic fibres and the postganglionic nerve cells

Blood Vessels

Around the endothelial cells of the capillaries and closely applied to them are found reticulin fibres on which are cells staining vitally with both methylene blue and trypan blue (Figs 6 17 18) According to the state of distension of the capillaries so the cells appear elongated along the length of a collapsed capillary or running transversely on a dilated capillary These cells correspond to the Pouget cells or pericytes Unmyelinated nerve fibres with attached Schwann cells can be

The satellite cells with which the Schwann cell syncytium is continuous have also been described as staining as macrophages (Conti 1946 Franceschini 1955) Schwann cells and satellite cells form macrophages in tissue culture (Weiss and Hsi Wang 1945) Cappell (1929) however could not confirm the vital staining of Schwann cells Weddell and Glees (1941–42) studying the degenerating peripheral portion of sectioned nerve fibres found that Schwann cells showed no reaction to vital dyes for 136 hours after nerve section However as pointed out above macro

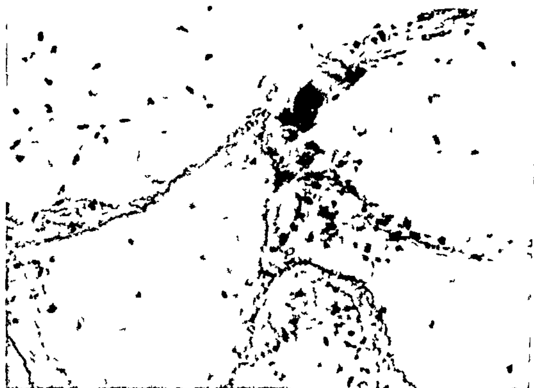


Fig 15 Subcutaneous tissue supravitally stained with methylene blue showing how macrophages are attracted round the unmyelinated nerve fibres (1 0)

phages may not take up vital dyes if they contain lipids as they will in the region of degenerating axons

Thus it appears that many epi peri and endo neural and the satellite and Schwann cells are of macrophage nature and that nerve fibres in the peripheral nerves pass through a syncytium of Schwann cells In the peripheral connective tissue unmyelinated nerve fibres pass near or are applied to the macrophages In the enteric visceral plexuses cells of similar nature are interposed between the axons and the plain muscle fibres of the gut wall as the interstitial or intercalary cells In the posterior root and the autonomic ganglia the ganglion cells are surrounded by a layer of satellite or capsular cells of macrophage nature lying on the reticulin of the capsule The dendrites of the ganglion cells form a dense network just beneath

to possess numerous delicate branches encircling the capillaries This situation of macrophages is possibly responsible for the frequent statement that macrophages occur in tissues chiefly perivascularly In inflammation the cells swell up become rounded display active movements and phagocytose bacteria and cellular débris (see Jaffe) as do other macrophages Fine unmyelinated nerve fibres accompanied by Schwann cells lying outside these cells and coming into close relationship with



Fig 19 Small blood essel of breast lying nea a yst wall showing el ng ted macrophages arranged circumfe enti lly around the essel wall nd beaded unmyelin ted nerve fib es with attached Schwann cells n l se lationsh p In the cyst wall l es a syncyt um of m crophages (Methylene blu × 00)

them were observed by St hr (1922 1939 see Fig o7 in Stöhr 1951) He thought however that the nerve fibres actually ended in these cells a condition I was unable to observe These nerve fibres are partly sympathetic and partly posterior nerve root in origin (Weddell Palmer and Paillie 1955)

Elongated and flat vitally staining adventitial cells with slender processes running parallel to the long axis of the vessels have been described in the adventitia of the small arterial vessels by many observers (see Jaffe) In the largest vessels such as the aorta macrophages are present throughout the different layers of the walls These take up sterol bodies such as cholesterol as in the condition of atheroma

(a) *Lung Alveoli* It is now generally agreed that the alveoli of the lungs are

observed immediately outside these cells but not apparently ending either on the pericytes or endothelial cells though in close relationship (Fig 18)

On larger arterioles replacing the pericytes are found elongated vitally staining cells which partly encircle the vessel wall They look like fibroblasts and may branch and their processes join with neighbouring members to form a syncytium of cells They lie in the adventitia and media between the fibroblasts and muscle



Fig 18 On left capillary with surrounding pericytes and unmyelinated nerve fibres with attached Schwann cells lying outside the pericytes A small nerve bundle is also shown on right (Methylene blue × 400)

cells and the connective tissue fibres They stain with both methylene blue and trypan blue (Fig 19) Running round the vessels and encircling them is a network of unmyelinated nerve fibres accompanied by Schwann cells in contact with the adventitia and also in some sections penetrating the walls These are in close relationship with but as far as can be made out with the limits of the ordinary microscope they do not make actual contact with the vitally staining cells

That pericytes are macrophages was reported by Veratti (1919) and Clark and Clark (1925) Cappell (1929) also states that perivascular macrophages are pericytes for example in the mesentery By silver impregnation they have been shown

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FIG 19 Small blood vessel of breast lying near a cyst wall showing elongated macrophages arranged circumferentially around the vessel wall and beaded unmyelinated nerve fibres with attached Schwann cells in close relationship. In the cyst wall lies a syncytium of macrophages. (Methylene blue × [illegible]00.)

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(*a*) *Lung Alveoli*. It is now generally agreed that the alveoli of the lungs are

lined by a layer of simple flattened epithelium the cells of which are united at their edges by a cement substance (Low 1953) Between these cells here and there smaller granular polygonal or typical branched cells (septal cells) are found These are the pericytes or macrophages of the alveolar capillaries The capillary walls are common to adjacent alveoli The macrophages or septal cells greatly increase in number and pass into the alveoli in inflammation or heart failure

(b) The *Glomeruli* of the kidney consist of a tuft of capillaries covered by visceral epithelial cells closely adherent to the capillary loops Between them is the basement membrane of the capillary These visceral epithelial cells are richly branched staining with Golgi s silver method (Zimmermann 1915–16) and according to him identical in character with the basket or myo epithelial cells of glands They stain with both methylene blue and trypan blue (Fig 39) They appear to be pericytes (macrophages)

Loose Connective Tissue

The macrophages in loose connective tissue appear as irregular star shaped or round cells or as elongated granular cells having the characteristics of fibroblasts The vitally staining cells seem to vary markedly in number in different sites and at



Fig 0 Bundle of ve fibres with Schwann cells approaching a number of f t cell Macrophages peel off the bundl of nerve fibres to surround th fat ells in th centre of the photograph Stain l vitally w th m thylene blue (× 00)

different times. When vital dyes are injected into the tissues, vital staining of macrophages appears especially around and close to epithelial and glandular structures, muscle fibres, capillaries and nerve fibres (Fig. 15) and is often confined to these regions. Not infrequently the macrophages of the connective tissue appear to peel off from the pericytes or from the perineural cells (Fig. 20). They can often be observed lying on reticulin fibres with their processes apparently attached to the latter. In other cases, for example in the loose connective tissue of ovary or uterine mucosa, the macrophages appear almost to replace the Schwann cells and the unmyelinated fibres to be applied to them (Fig. 16).

The *omentum and serous membranes* consist of loose connective tissue composed of collagenous fibres and fat covered by a layer of mesothelium lying on a basement membrane. In the connective tissue below the latter methylene blue staining shows long rows of macrophages lying between collagen fibres and also blood vessels and unmyelinated nerve fibres. The macrophages may be collected together as dense masses or milky spots.

Collagenous Connective Tissue

Between the fibres of collagenous connective tissue, in addition to the fibroblasts, can be seen cells which stain vitally with both methylene blue and trypan blue. They have various shapes, often spindle shaped and having the typical appearance of fibroblasts, often with branched processes forming a syncytium or rounded off. They are frequently found as long rows of cells, the processes of which seem continuous. In places they are seen to be continuous with the pericytes of the capillaries. The macrophages are distinct from the fibroblasts between which they lie. Fine unmyelinated nerve fibres can be seen in places running between the collagen fibres in relation to these macrophages.

Tendon

In this tissue the appearances are the same as those observed in collagenous connective tissue or aponeuroses. Cells lying between the collagen fibres correspond to those known as tendon corpuscles, usually considered as fibroblasts. They are compressed into various shapes by the surrounding fibres. Some of these stain vitally, appear continuous by their processes with one another and can be seen to be derived from and continuous with the pericytes of the capillaries (Fig. 21). Unmyelinated nerve fibres can be observed in relation to the capillaries and also running between the collagen fibres in the proximity of the tendon corpuscles. At the junction of the tendon with muscle the reticulin and collagen fibres become continuous with the sarcolemma of the muscle fibres and the vitally staining corpuscle syncytium with similar cells lying everywhere between the muscle fibres and on the sarcolemma (see below).

Fat

Normal fat consists of fat cells bound together by reticulin fibres into lobules. Each individual fat cell is completely surrounded by a reticulin covering. The lobules are held together and divided from one another by septa consisting of fibroblasts

and reticulin and collagen fibres In these septa lie blood vessels and capillaries and fibroblasts branch off to run in the reticulin framework dividing the fat cells from one another By vital staining with either methylene blue or trypan blue there can be observed numerous macrophages forming a syncytium lying on the reticulin framework (Figs 5 6 22) They may be round spindle shaped or stellate or wrap round the fat cells In some sections it can be seen that these macrophages are in fact continuous with the pericytes of the capillaries In the connective

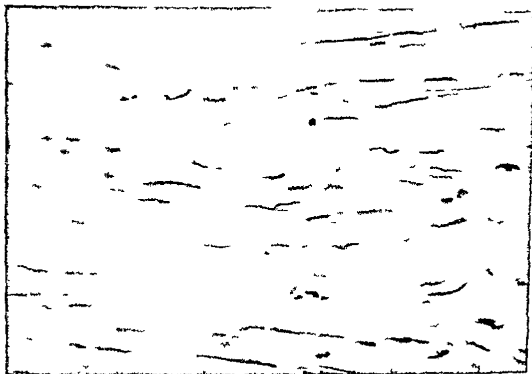


Fig. 21 Vitally stained tendon showing uptake of dye by some tendon corpuscles (Methylene Blue × 200)

tissue septa between the lobules can be seen macrophages continuous with those between the fat cells and also larger blood vessels with accompanying nerve fibres Unmyelinated nerve fibres accompanied by blood vessels may also be observed running in these septa in close relationship to the macrophages Very fine nerve fibres are present along the capillaries and lying between individual fat cells (Figs 5 22)

Maximov (1927) also describes and portrays vitally staining resting wandering cells around and in close relationship to fat cells These cells are those described as interstitial cells by Boeke The typical reticulo histiocytic structure of fat was shown by Volterra (1921–23) Wassermann (1926) Dogliotti (1928) Goldner (1936) Hett (1938) and Aloisi (1939) Kure *et al* (1937) describes the termination of un

myelinated fibres in relation to subcutaneous fat cells. Unmyelinated fibres were also demonstrated in the vicinity of these interstitial cells in adipose tissue by Boeke (1939–40).

Meninges

The *dura mater* consists essentially of collagenous connective tissue and stains like that elsewhere. The macrophages likewise occur perivascularly but are especially obvious at the points of exit of the peripheral nerves. On the surface of

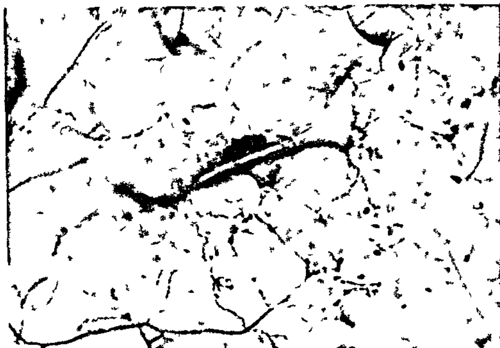


FIG. Normal fat showing fine beaded unmyelinated nerve fibres lying between fat cells. (Methylene blue × 200.)

this membrane there is found a thin layer of loose connective tissue containing collections of macrophages and fibroblasts continuous with those between the collagen fibres proper. Unmyelinated nerve fibres are found as in other collagenous connective tissue.

Cappell (1929) also found that the dura contained many macrophages, especially perivascularly and at the points of exit of the peripheral nerves.

The *pia arachnoid* is composed essentially of loose connective tissue formed of interlacing reticulin and some collagenous fibres and containing numerous blood vessels of various sizes. Fat cells may also be present. Macrophages are especially numerous around the pial blood vessels. The mesothelial cells covering the surface of the pia arachnoid also take up vital dyes to some extent. Numerous unmyelinated

nerve fibres are found especially perivascularly but also in the connective tissue itself

Cappell (1929) describes similar findings Woollard (1923-24) showed that in conditions of irritation in the presence of particulate foreign matter the arachnoid mesothelial cells become mobilized in the form of free macrophages and react in a characteristic manner with vital dyes

The *choroid plexus* consists of a layer of pia arachnoid covered by an epithelium derived from the ependymal cells lining the ventricles Very numerous macrophages and a heavy concentration of reticulin lies beneath the epithelium (see Franceschini 1935)

Striped Muscle

Striped muscle is formed of parallel muscle fibres held together by connective tissue The arrangement in muscle is similar to that in tendon Here as with the collagen bundles in tendon the muscle fibres combine to form primary bundles and several of these to form secondary bundles and these again into tertiary bundles

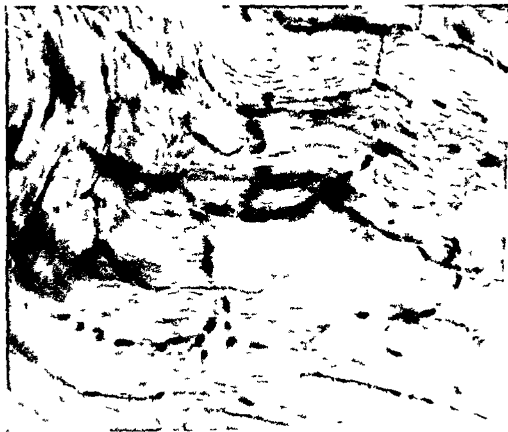


FIG 3 Striped muscle vitally stained with methylene blue showing macrophages lying in relation to sarcolemma and continuous with pericytes of capillaries Unmyelinated nerve fibres pass in close proximity to the macrophages (× 400)

etc The layers of interstitial connective tissue at the periphery of the muscles the epimysium project into the spaces between the bundles of muscular fibres as the perimysium This consists of irregularly arranged collagenous reticulin and elastic fibres and many varieties of connective tissue cells including fat The perimysium sends thin layers between the smaller bundles and between separate muscle fibres as the endomysium As in smooth muscle this consists of thin reticulin networks which form capsules for the fibres The endomysium also contains connective tissue cells which play an important role in inflammation (Maximov and Bloom) and numerous capillaries around the individual muscle fibres Immediately surrounding the muscle fibres is the sarcolemma a structureless transparent reticulin membrane completely investing the fibres and comparable with a basement membrane

Experimentally it was found (as other observers have done) that muscle fibres do not take up vital dyes or methylene blue unless damaged The fibres are surrounded by a reticulin membrane on which are found rows of macrophages staining with both methylene blue and trypan blue (Fig 23) The muscle fibres are completely encircled by these cells which are joined together by their processes In places they are seen to be continuous with the pericytes of the capillaries Where muscle is attached to tendon or aponeurosis these macrophages become continuous with similar cells between the collagen fibres of the latter (tendon corpuscles) Between the muscle fibres and in close relationship with the vitally staining macrophages are found unmyelinated nerve fibres as in tendon

Volterra (1927) noted the presence of macrophages in relation to the sarcolemma Cappell (1929) also describes them in the connective tissue between muscle fibres closely applied to the capillary walls in some places and in others forming an almost continuous chain of cells between the muscle fibres and around large vessels and nerves Where connective tissue is more abundant the cells were also more numerous so called adventitial cells or myocytes Jaffe (1938) Maximov and Bloom (1952) and Franceschini (1955) also mention the occurrence of macrophages lying between muscle fibres on the reticulin network Boeke describes these cells but as interstitial cells The presence of numerous unmyelinated nerve fibres some of posterior nerve root origin with free nerve endings between striated muscle fibres has been frequently demonstrated for example by Feindel Weddell and Sinclair (1948) and by Boeke

Where a muscle is attached to a tendon there is a close union of the muscle fibres with the collagenous bundles of the tendon The collagenous bundles of the perimysium pass directly into those of the tendon and the sarcolemma likewise fuses with the ends of the collagenous bundles

Cardiac Muscle

The structure of cardiac muscle is the same as that of striped muscle in respect of its macrophages All muscle and conducting fibres are surrounded by a covering of macrophages derived from pericytes (Figs 8 9) Nerve fibres were found in close relationship to the macrophages but did not appear to end on the muscle fibres In the endocardium and pericardium the picture is that of loose connective tissue

nerve fibres are found especially perivascularly but also in the connective tissue itself

Cappell (1929) describes similar findings Woollard (1923-24) showed that in conditions of irritation in the presence of particulate foreign matter the arachnoid mesothelial cells become mobilized in the form of free macrophages and react in a characteristic manner with vital dyes

The *choroid plexus* consists of a layer of pia arachnoid covered by an epithelium derived from the ependymal cells lining the ventricles Very numerous macrophages and a heavy concentration of reticulin lies beneath the epithelium (see Franceschini 1935)

Striped Muscle

Striped muscle is formed of parallel muscle fibres held together by connective tissue The arrangement in muscle is similar to that in tendon Here as with the collagen bundles in tendon the muscle fibres combine to form primary bundles and several of these to form secondary bundles and these again into tertiary bundles



FIG 23 Striped muscle vitally stained with methylene blue showing macrophages lying in relation to sarcolemma and continuous with pericytes of capillaries Unmyelinated nerve fibres pass in close proximity to the macrophages (× 400)

devour bacteria They are demonstrable with silver impregnation like other reticulo endothelial cells The contained lipid droplets are dissolved out in paraffin (hence Gitter or lattice cells or compound granular corpuscles) They occur perivascularly as pericytes and similar cells are seen in the adventitia of larger arterial vessels and as perineural satellites and form part of the glial reticulum They lie in relation to the glial fibres which support the neural elements The cells do not stain with acid colloidal dyes in the resting state but do so after stimulation or injury For this reason acid colloidal dyes injected intravenously do not stain the brain unless it has been injured In many pathological processes for example trauma infection or hæmorrhages the microglial cells retract their processes and the nucleus becomes elongated or rod shaped (rod cells) and is found thus in many chronic degenerative conditions of the central nervous system They take up debris and may form multinucleated giant cells

When methylene blue is injected directly into central nervous tissue staining of macrophages lying between the fibres and around the nerve cells is observed They are seen clustered around the capillaries from which they seem to diverge to pass between the fibres as parallel rows and to lie around the ganglion cells In addition plentiful unmyelinated fibres are also stained as they lie between the myelinated fibres and obviously they are in close relation to the numerous microglial cells The picture closely resembles that found in striped or cardiac muscle

The Eye

A description of vitally staining structures in the eye is given by Moraud (1934) The pericytes of the choroid were studied by Wolter (1956) The connective tissue coverings of the optic nerve are continuous with the meninges and consist of collagen and reticulin fibres accompanied by numerous macrophages In the ciliary body again very numerous macrophages are present especially perivascularly as pericytes In the iris which is largely of mesenchymal origin there is abundant reticular tissue pigment cells and again numerous macrophages especially perivascularly The sclera and episclera which are connective tissue structures are composed of collagen and reticulin fibres and macrophages are everywhere present In the choroid reticular fibres and macrophages are also found throughout all the layers including that just below the pigment layers of the retina The choroid is divided from the sclera by the supra choroid layer a loose pigmented tissue in which the melanoblasts are situated Throughout this tissue reticulin fibres fibroblasts and numerous macrophages containing pigment are present

The retina and optic nerve which are of neural origin contain microglial cells which behave as do those in the central nervous system

The *cornea* is of epidermal origin and consists of fibrous connective tissue continuous laterally with the sclera It is composed of regular laminæ of fibres between which lies a network of flattened cells (corneal corpuscles) branched and united by their processes The corneal corpuscles store dyes in vitally stained new born animals (Maximov and Bloom 1952) When the cornea is inflamed the corpuscles become mobile and actively phagocytic According to Maximov and Bloom nerves pass into the cornea and form a plexus the fibres of which become lost in the corneal

A layer of macrophages lies on the basement membrane below the covering endothelial cells Nerves blood vessels and fat cells are also present in this situation

Cappell (1929) and others (see Jaffe 1938) also describe macrophages lying between cardiac muscle fibres the picture being similar to that of striped muscle They have been termed myocytes Mitchell (1956) reports interstitial ' cells as lying between the muscle fibres and related to the nerve fibres Collections of macrophages have also been described lying immediately beneath the endocardial lining cells of both valves and heart chambers and the surface of the pericardium (Pfuhl 1929 1931)

There is no general agreement regarding the terminal distribution of nerves to the cardiac muscle fibres (see Kuntz 1953 Mitchell 1956) All workers describe a subepicardial plexus from which fibres arise to penetrate and ramify throughout the myocardium forming a loose meshwork between fascicles of muscle cells and especially around conducting fibres Some have described them as ending on the myocardial cells others have denied this and considered the fibres as affecting the conducting fibres though the mode of ending is unknown In view of this uncertainty and my own observations it may well be that they end freely in the connective tissue in relation to the macrophages as they do in striped muscle

Smooth Muscle Fibres

In the wall of the gut uterus and bladder the bundles of smooth muscle fibres are surrounded by connective tissue containing macrophages The reticulin fibres and capillaries of the connective tissue continue into the spaces between the individual muscle fibres and surround each with reticulin and cells which stain vitally both with methylene blue and trypan blue and which are thus macrophages (Fig 55) They are often compressed and elongated to a spindle shape like fibroblasts or have a stellate or other form They are continuous by their processes to form a syncytium These cells correspond to the interstitial cells of Cajal intercalary or Kölliker cells *Unmyelinated nerve fibres are in close relationship to the cells* In the media of small arteries the muscle fibres lie between elongated vitally staining cells arranged circumferentially around the vessel as the adventitial cells in close relationship to unmyelinated fibres (Fig 19) In the pilomotor muscles of the skin similar vitally staining elongated cells are seen between the muscle fibres

The Central Nervous System

The central nervous system consists of nerve cells and their dendrites and axons lying in the neuroglia which acts as a supporting and nutritive medium and contains three types of cell astrocytes oligodendrocytes and microglial cells

Microglial cells are found throughout the nervous system Similar cells are found in the optic nerves and retina They have been given various names such as compound granular corpuscles Gitter or lattice cells foam cells lipophages etc They are seen as small angular and irregular shaped supporting cells with numerous branching processes forming a syncytium Flat cells resembling endothelium and lamellar forms in the narrow interstices between the surfaces of nerve fibres are also seen They store lipid substances cellular debris iron pigment dyes etc and also



Fig 4 Perichondrium showing macrophage syncytium and unmyelinated nerve fibres in contact with cartilage below and fat above (Methylene blue × 00)

Epithelia

With all epithelia satisfactory staining with methylene blue is difficult to obtain owing to rapid decolourization A solution of 0 06% of methylene blue and hydrogen peroxide gives the best results

(a) *Respiratory epithelium of trachea bronchi and bronchioles*

The epithelial cells lie on a basement membrane composed of reticulin in which can be observed numerous macrophages Other macrophages lie in the submucosal connective tissue Vital dyes tend to condense on and stain the basement membrane Between the epithelial cells and with one process attached to the basement membrane are found numerous vitally staining thin elongated cells lying perpendicular to the membrane and dividing the epithelial cells from one another Some of these cells appear to have rounded off and to have passed on to the surface of the mucosa or are in process of doing so Numerous fine unmyelinated nerve fibres can be observed lying below and in the basement membrane and running between the epithelial cells but they do not appear to end in these (Fig 25) The bronchial glands stain like other glands as described below (Fig 26)

Lubarsch (1921) described the occurrence of macrophages in the loose connective

cells which appear to replace the Schwann cells Weddell and Zander (1950) and Zander and Weddell (1951) using vital methylene blue staining found that nerve axons entered the cornea and coursed as networks between the corneal fibres of the substantia propria and along the surface of the cytoplasm of a longitudinal series of Schwann cells continuous by their processes with neighbouring cells Simple axons leave these bundles and networks and proceed without Schwann cells for some distances as free beaded *nerve endings Some of these end between the cells of the* corneal epithelium and others in relation to the corneal corpuscles which seem to take the place of the Schwann cells These nerve fibres are of course posterior or trigeminal sensory root in origin Thus the corneal corpuscles are macrophages forming a syncytium and appear to take the place of Schwann cells in the peripheral nerves Nerve fibres pass into relationship with the constituents of the syncytium Zander and Weddell add that the plexiform arrangement of nerve fibres in the cornea suggests that the fibres perform other functions than subserving sensation for example that of nocifensor nerves

Synovial Membranes

These consist of a layer of synovial lining cells lying on a collagenous connective tissue containing fat Macrophages are found throughout these structures and especially below the layer of secreting synovial cells next to the joint cavity (see also Rynearson 1931) *Unmyelinated nerves accompany blood vessels* and end in a terminal arborization in the layers beneath the surface mesothelium in close relationship to the macrophages (Maximov and Bloom 1952) In the proliferated and œdematous synovial fringes of cases of arthritis the whole central core within the lining synovial cells consists of very numerous macrophages on reticulin fibres (see Franceschini 1955)

Periosteum and Perichondrium

These were examined in the long bones of mice stained vitally with methylene *blue and trypan blue and in the perichondrium of the bronchial cartilages in human* material obtained from pulmonary lobectomy and pneumonectomy specimens The membranes were similar in structure and consisted essentially of an external layer of vitally staining macrophages of various shapes some looking like fibroblasts joined by their processes to form a syncytium and with unmyelinated nerve fibres running in close relationship *These cells lay* on and among *reticulin* and *collagen* fibres and fibroblasts Deep to these were non staining chondroblasts or osteo blasts lying on the surface of the cartilage or bone respectively (Fig 24)

The endosteum lining the cavity of the bone marrow is like the periosteum composed of reticulin and collagen fibres fibroblasts and also numerous macrophages Cappell (1929) also found vitally staining *cells* in the endosteum *He also describes* the presence of free macrophages in close apposition to the bony lamellae in the developing bone of young animals Many of the unmyelinated nerve fibres of the periosteum are of posterior nerve root origin and conduct impulses giving rise to diffuse compelling pain

tissue of the respiratory mucosa and their situation just below the epithelium and perivascularly Macrophages are frequently seen in or traversing this epithelium according to Franceschini (1955) Strelin (1929) also records the existence of special cells with phagocytic properties and capable of amœboid movement lying in the epithelium Feyrter (1953) describes typical Hellen Zellen lying on the basement membrane of this epithelium with their processes between the epithelial cell All these cells correspond with those observed in my own preparations

Kuntz (1953) states that a small subepithelial nerve plexus is found in the trachea and bronchus Nerve fibres are found below and in the bronchial epithelium and in that of the nose Many of these are of visceral sensory that is posterior nerve root origin Buchner (1944) and Fröhlich (1949) showed the relationship of the clear



Fig Inflamed bladder mucosa supravitally stained with methylene blue showing macrophages in basement membrane and lying perpendicular to this between epithelial cells Beaded unmyelinated nerve fibres are also present between the epithelial cell (× 00)

Hellen Zellen cells to terminal neurofibrils which they thought ended in the cells In the respiratory epithelium of the nose nasal sinuses and Eustachian tube typical Hellen Zellen (macrophages) are found lying on the basement membrane below the epithelial cells (Feyrter 1953) They are also seen in the epithelial covering and interstitial tissue of nasal polyps

(b) Transitional epithelium of the pelvis of the kidney ureter bladder and urethra

The macrophage distribution in the mucosa of the bladder urethra ureter and pelvis of the kidney is similar to that in the bronchial mucosa A condensation of macrophages is found in the basement membrane and thin elongated vitally staining cells perpendicular to and fixed to the basement membrane are observed lying between the epithelial cells Some of these appear to have rounded off and to be passing on to the mucosal surface Unmyelinated nerve fibres run parallel to and ramify in the basement membrane and pass up between the epithelial cells They do not appear to end in these cells but lie freely between them (Fig 27)



FIG 5 Bronchial epithelium supravitally stained with methylene blue showing macrophages *lying in the basement and between epithelial cells* Fine *beaded* unmyelinated nerve fibres are found in the basement membrane within the epithelium and in the submucosa (× 400)

FIG 6 Bronchiole Transverse section showing macrophages and nerves in the basement membrane and between the epithelial cells supravitally stained with methylene blue (× 00)

are obtained with trypan blue indicating their macrophage nature These correspond to the interstitial cells The cells often show a vacuolated appearance and in the small intestine appear to contain fat droplets In the connective tissue of the villi are found capillaries surrounded by macrophages and unmyelinated nerve fibres from the submucous plexus form a plexus completely surrounding the villi and lying in close relationship to the macrophage syncytium of the basement membrane and between it and the pericytes of the capillaries With either stain there may occur staining of elongated macrophage cells lying between the epithelial cells with their long axes at right angles to the basement membrane The argentaffin or Kultschitzky cells do not stain vitally

Macrophages lying between the intestinal epithelial cells were described by Arnstein (1867) Schafer (1885) Clark and Clark (1916–17) and Leach (1938) Some migrate into the lumen of the gut Others occur below the epithelial cells around and lining the walls of the lacteals and in the cells of Meissner s plexus (see Leach 1938) Macrophages lying immediately beneath the basement membrane are described by Schaffer (1927) and portrayed by Maximow (1927) These take up pigment in cases of melanosis of the intestine Cappell (1929) also reports the presence of vitally staining cells in the connective tissue and submucosa in the stroma of the mucosa and villi and in lymph follicles Cajal Boeke and others have of course described interstitial cells lying in the submucosa the macrophage nature of which has been shown According to Spoerri (1947) the processes of the interstitial cells of Cajal make direct contact with the gastric epithelial cells of all types including both the glandular and surface epithelium Franceschini (1955) regards the argentaffin cells as macrophage in nature but they do not appear to stain vitally and their function as neuro secretory cells has recently been elucidated A subepithelial nerve plexus has also been described by Hill (1927) and Waddell (1929) It ramifies in the basement membrane and between epithelial cells

(d) Uterine epithelium

The results of vital staining vary to some extent with the age of the subject and the phase in the human menstrual cycle at which the specimen is obtained In specimens taken midway between the menstrual periods very numerous macrophages are found in the connective tissue of the whole mucosa In the epithelium itself macrophages lie in the basement membrane and form a syncytium while others are found lying perpendicular to the membrane between the epithelial cells They are found condensed around the capillaries and the basement membranes of the glands as they dip downwards In the latter situation as seen in cross section they appear as a syncytial collar around the tubular glands Other macrophages lie perpendicular to the basement membrane between the gland cells Unmyelinated nerve fibres can be found in the deeper layers of the mucosa lying closely applied to the macrophages on the basement membrane (Fig 29) During the reparative stage of the menstrual cycle large numbers of macrophages can be made out in the denuded part of the mucosa In the senile uterus the glands are largely atrophic and the mucous membrane thinned Many fewer macrophages are demonstrable in the connective tissue They are scattered throughout the tissue however

These same macrophage cells were also described by Feyrter (1953) as Hellen Zellen, occurring in various shapes—three cornered flask like round or prismatic with fine granules and vacuoles and giving the typical staining of these cells Similar cells are seen in the submucosa According to Feyrter and as one would expect with macrophages the Hellen Zellen in the bladder mucosa are particularly prominent in cystitis *Hellen Zellen lying in the submucosa also vary in number* with the degree of inflammation In the bladder nerve terminations in the mucosa and submucosa were described by Langworthy and Murphy (1939) Fibres penetrate the epithelium and lie between the cells as varicosities These fibres are of sensory nature that is of posterior nerve root origin

(c) Gastro intestinal epithelium

The epithelium of the gut from stomach to rectum consists of columnar cells lying on a basement membrane It is of course markedly folded to form the villi and glands When using a supravital stain for the mucosa it is essential to wipe away all mucus in order to stop the dyes from being taken up by the mucus and thus preventing vital staining Injections are made immediately beneath the epithelium Using 0 06% methylene blue solutions the preparations show a concentration in the basement membrane of the mucosa in which lies a syncytium of vitally staining cells completely surrounding the crypts and acini (Fig 28) Similar appearances

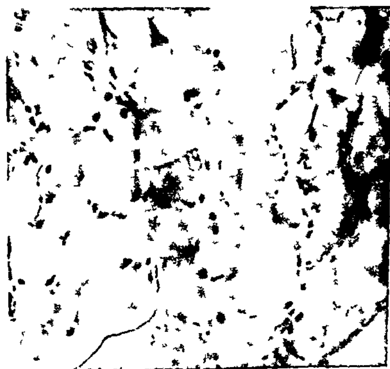


Fig. 28 Mucosa of large gut Transverse section of crypt supravitally stained with methylene blue showing macrophages lying on basement membrane and beaded unmyelinated nerve fibres in close relationship to them (× [illegible]00)

spayed animals There is an enormous increase in the numbers staining as a result of administration of sex hormones (Hofbauer 1926 Fluhman 1928 1932 see Jaffe 1938) The macrophages of the unstriped mu cle layers of the uterus resemble those of the gut etc

Brown and Hirsch (1941) in the immature human uterus showed an intricate unmyelinated nerve plexus in the lamina propria and fibres in relation to the uterine epithelium In the adult some observers have traced nerves into the mucosa and in relation to the epithelium In view of the prolonged degenerative and regenerative changes occurring the existence of nerve fibres terminating in the epithelium must be con idered doubtful State and Hirsch (1941) observed nerves throughout the basal one third of the endometrium related to arteries but also some ending freely in the stroma There was no evidence of their ending in relation to epithelial cells (Kuntz 1953) The mode of ending of uterine mucosal nerves does not seem to be known definitely (Maximov and Bloom 1952)

Thus in the immature and senile uterus macrophages are found between the epithelial and gland cells and in the ba ement membranes Unmyelinated nerve fibres are in close relation hip to the cells in the submucosa and ramify in relation to the basement membranes During the menstrual changes many macrophages are found in all layers of the mucosa but the existence of nerve fibres in the more superficial layers is doubtful

Exocrine Glands

Using vital methylene blue staining in exocrine glands including the breast sweat salivary sebaceous pancreas and prostate the bronchial glands and their ducts and also in the testis and epididymis and its ducts and the seminal vesicles the results are similar (Figs 1 2 3 4 29–35) Surrounding and lying in or on the reticulin basement membrane of the acini or ducts is found a collection of vitally staining cells having a stellate appearance and apparently joined by their processes These completely surround the acini forming an open network Using trypan blue staining the same syncytium of cells can be observed to take up the dye A similar syncytium surrounds the tubules of the testis and the ducts of the epididymis (Figs 33–34) The glands of the uterine mucosa are also wreathed by macrophages lying in the basement membrane (Fig 29) As with epithelia macrophages are observed lying on the basement membrane and projecting towards the lumen of the acini between the acinar cells With methylene preparations the acini can be seen to be surrounded by unmyelinated fibres with attached Schwann cells They formed a close network around the acini and came into close relationship with the basement membrane of the acini and ducts but on no occasion in examining hundreds of sections were the nerve fibres ob erved to end in the macrophage syncytium though they coursed in very close relationship to this Often processes of the branched cells were stretched out towards the nerve fibres They were however always divided by a small but definite space from them Surrounding the acini are also observed capillaries accompanied by pericytes staining vitally In the interstitial tissues nerves are found around the blood vessels In many ca es

especially around the capillaries as pericytes They are also found in the basement membrane of the surface epithelium and that of the glands and between the epithelial and gland cells Unmyelinated nerve fibres are now found in profusion throughout the mucosa some lying in or near the basement membrane of the surface epithelium and glands and others in close relationship or applied to those in the connective tissue where the macrophages seem to serve as Schwann cells for the fibres

Many workers have reported that in the uterus Fallopian tube and vagina

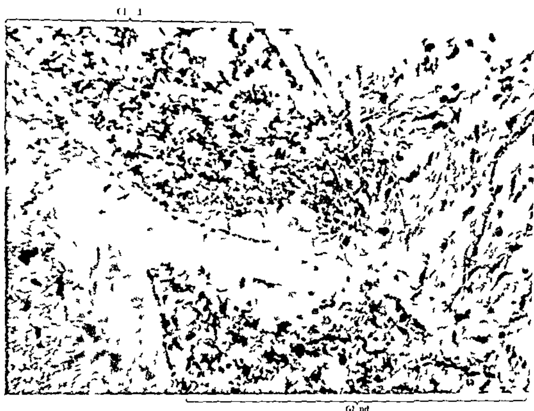


Fig 9 Uterine glands in deeper part of mucosa vitally stained with methylene blue showing macrophage syncytium lying in basement membrane and beaded fine unmyelinated nerve fibres coming into relationship with macrophages (× [illegible]00)

macrophages are present throughout the various layers They are found especially around the blood vessels Cappell found numerous macrophages in the deeper layers of the endometrium Typical Hellen Zellen are reported on the basement membrane of the surface epithelium and in the acini of the glands (Feyrter 19[illegible]3) During pregnancy the number of functioning macrophages appears to increase enormously in all layers of the uterus and Fallopian tubes and also in the lateral ligaments of the uterus They persist for a long time after parturition They also increase as a result of aseptic inflammation produced by various means such as local stimulation They are difficult to show and are reported as practically absent in the uterus of

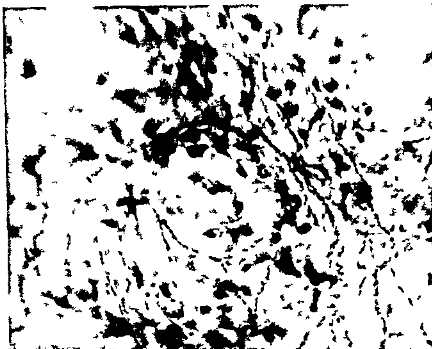


Fig 3 Irostat a ini lowing ma rophage yncytium surround ng th m and fine un myel nated n ve fibres coming into cl se el tion h p (Methylene blue × 00)

Fig 33 Tubul of t t s surrounded by ma phag syn yt um sh wing nerve fibr s in lo el t on h p (Methylene bl × 00)

FIG 30 Tangential section of large acinus of breast vitally stained with trypan blue showing macrophages with fine processes coming into contact with their neighbours and forming a syncytium (× 400) A similar syncytium is found around all exocrine gland acini

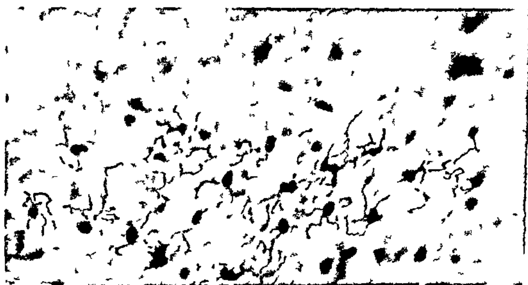


FIG 31 Wall of acinus of a sebaceous gland vitally stained with trypan blue showing syncytium of interstitial cells and indicating their macrophage nature (× 400)

enormous numbers of very fine unmyelinated nerve fibres can be seen in relation to the macrophages of the connective tissue for example in the prostate

The occurrence of vitally staining cells in the basement membrane of glands is mentioned in passing by Schaffer (1927) Maximov (1927) also remarks on the presence of macrophages around the basement membrane and between the acini (the so called Zwischenzellen) of the salivary glands Volterra (1927) reports the existence of flattened or elongated macrophages in all basement membranes Franceschini (1929) remarks on their presence in the basement membrane of the choroid plexus According to Cappell (1929) in the interacinous tissue of the pancreas and salivary glands macrophages are found closely applied to the basement membrane of the acini and their processes extend between neighbouring acini They lie between the capillary endothelium and gland cells The occurrence of macrophages around blood vessels and below the basement membrane of the testicular tubules was described by Stieve (1930) The syncytium of vitally staining cells around the acini and ducts correspond in their situation general appearance and phagocytic properties and general characteristics with the interstitial cells of Cajal those between the gland cells with the myo epithelial or basket cells Hellen Zellen were described in the same situation in salivary sebaceous sweat mammary prostatic and pancreatic glands and in the ductus epididymis and seminal vesicles by Feyrter (1953)

It has been shown that in the mucosæ of the respiratory and urinary tract the uterus and the gut and in all exocrine glands there are demonstrable macrophages lying in the basement membrane but also lying between the epithelial cells perpendicular and anchored to the basement membrane Some of these cells may become detached and pass into the lumen of the organ especially during inflammation On all epithelial surfaces and in all glandular acini and ducts such macrophages are often described as traversing the epithelium for example in the bronchi tonsillar epithelium seminiferous tubules epidermis gut breast sebaceous salivary and lachrymal glands and the epithelium of the choroid plexus This phenomenon is known as histiocytic assistance of the epithelia and is thought to be concerned with epithelial or gland cell activity in some way (see Franceschini 1955) The cells however are not traversing the epithelia but are fixed macrophage elements with their basal processes on the basement membrane from which they may become detached The temporary transepithelial migration of macrophages may be seen in the ducts of mammary glands after injection of thorotrast into the ducts (Osselladore 1937) of pyrrol blue into the ducts of salivary glands (Staudacher Dalle Aste and Bellinazzo 1947) or of contact of Chinese ink with tonsillar epithelium (Taillens 1944) After migration to the surface of the tonsil or lumen of the ducts the macrophages filled with the foreign substance retraverse the epithelium and return to the original site in the basement membrane or outside the excretory duct The same phenomena are observable in the absorption of fat by the small intestine (Leach 1938)

The question of the relationship of nerve fibres to the gland cells and the cells

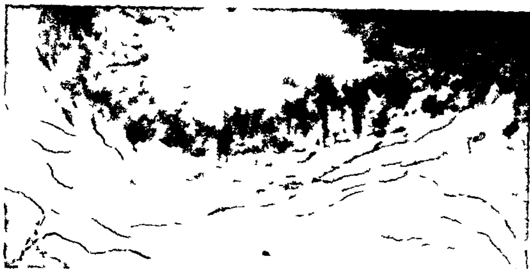


Fig 34 Tubule of testis showing vitally staining macrophages lying in the basement membrane and between the Sertoli cells (Methylene blue × 400) Unmyelinated nerve fibres come into close relationship with the former



Fig 35 Acini of sweat gland vitally stained with methylene blue showing macrophages around basement membrane and beaded unmyelinated fibres in close relationship (× 400)

to the comparatively poor diffusibility of the former The cells are identical with the cells of Langerhans of the upper layers and the clear cells of Masson of the basal layers of the epidermis as described above Unmyelinated fibres accompanied by Schwann cells run up from the dermis and then parallel with the basement membrane for a short distance They then pass upwards between the epidermal cells In some cases they seem to have Schwann cells attached even within the epidermis where they come into close relationship with the processes of the cells of



FIG 36 Skin vitally stained with methylene blue showing vitally staining clear and Langerhans cells in the epidermis with fine unmyelinated nerve fibres lying in close relationship to their processes (× 00)

the vitally staining syncytium Sometimes the fibres seem almost to continue into the processes of these cells These fibres are of course the slow conducting unmyelinated C fibres of the posterior nerve roots In *hair follicles* the same arrangement of unmyelinated nerve fibres clear cells and cells of Langerhans is observable in the outer hair sheath (Figs 12 13)

The close relationship of Langerhans cells to nerve endings in the epidermis has been remarked on by numerous observers including Langerhans himself and Masson In hair follicles Weddell Palmer and Paillie (1955) found evidence in favour of independent sets of stem axons and free nerve terminals situated one within the other The outer set circled the hair among the cells of the middle layer of the

of epithelia is a much discussed problem which has already been touched on Without entering the lists on one or other side in this controversy it may be stated that in all these glandular organs a plexus of nerve fibres closely surrounds the acini and ducts According to Maximov and Bloom (1952) nerve fibres passing to glands may form a plexus below and then penetrate and form a plexus above the basement membrane In regard to the seminal vesicles the existence of branches penetrating the basement membranes into the epithelium is doubtful (Maximov and Bloom 1952 Kuntz 1953) Kuntz summarizes the present knowledge on the innervation of the salivary glands and breast In the former the entering nerves are closely associated with blood vessels and the ducts within the glands Most of the nerve fibre bundles lie in proximity to interlobar and interlobular ducts and arteries Peri alveolar plexuses are also found Nerve fibres in relation to mammary gland cells have not previously been demonstrated anatomically though fibres have been shown to terminate in relation to blood vessels and smooth muscle Physio logical data however indicate the existence of nervous control of these glands The presence of periacinar plexuses was readily demonstrated however in my own preparations *Weddell Palmer and Pallie (1955) found that in sweat glands it is* difficult to demonstrate the ending of nerve fibres but some stem fibres give rise to profuse arborizations of fine axoplasmic filaments which terminate freely in rela tion to myo epithelial cells others give rise to a profusion of filaments which terminate freely in and between the secretory cells of the glands

It appears therefore that in epithelia and glandular acini and their ducts and *in the testis epididymis and seminal vesicles a plexus of nerve fibres lies in close* relationship to the basement membranes On or in the latter lies a syncytium of cells staining vitally with methylene blue Janus green B and trypan blue and which are demonstrable with reduced silver and gold methods of staining These cells appear to correspond to the interstitial cells Similar vitally staining cells appear attached to the basement membrane and project between the gland cells They seem identical *with the basket or myo epithelial cells and the Hellen Zellen of Feyrter* These cells are in fact macrophages Unmyelinated nerve fibres from which neuro hormones may be liberated come into close relationship with these phagocytic cells

Epidermis

Vital staining with methylene blue is best obtained with 0 04% solutions injected *immediately beneath the epidermis The loose tissue beneath the epidermis contains* numerous macrophages Dye tends to concentrate on the reticulin of the basement membrane and between individual cells of the epidermis and the outer hair sheaths This may represent an extension of the reticulin between the epidermal cells Between the normal cells of the epidermis and present throughout the epidermal layers are found numerous cells staining vitally with methylene blue trypan blue and Janus green *B (Figs 10 11 12 13 30) Similar cells are found lying in the basal layers of* the epidermis The cells usually have a stellate appearance with the processes of neighbouring cells continuous or in close apposition to form a syncytium In some cases however the cells appear round or ovoid It is more difficult to stain the cells with trypan blue or Janus green B solutions than with methylene blue owing

were found to take up both methylene and trypan blue dyes and are in fact macrophages The evidence points to the fact that the stellate reticulum is composed of macrophages

During the development of the teeth the formation of dentine depends on the activity of the odontoblasts Vital staining of macrophages in relation to the odontoblasts has been reported by many workers (see Lehner and Plenk)

The dental pulp consists of embryonic connective tissue with abundant gelatinous basophil ground substance and collagenous and reticulin fibres It contains numerous macrophages In the pulp a plexus of unmyelinated nerve fibres is found with endings described as lying between and in the odontoblasts (Kuntz 1953) These are the unmyelinated posterior nerve or trigeminal sensory root fibres

In the fully formed teeth odontoblasts and macrophages remain adjacent to dentine in the pulp cavity When dentine is denuded or the outside of the tooth irritated a production of new or secondary dentine may be observed on the wall of the pulp cavity and may fill it Odontoblasts play a role in the nutrition of dentine Dentine is sensitive to touch to cold acid containing food etc However only occasional nerve fibres penetrate the dentine and extend for short distances

In the teeth therefore the cells of the stellate reticulum of the enamel organ are macrophages In the dentine and pulp the odontoblasts are accompanied by macrophages near or in which are unmyelinated nerve fibres of the pulp

Thyroid Gland

Around the acini of the gland a syncytium of vitally staining stellate or ovoid cells is found lying in the basement membrane (Fig 37) The individual cells are in contact by means of their processes Similar vitally staining cells are found in the

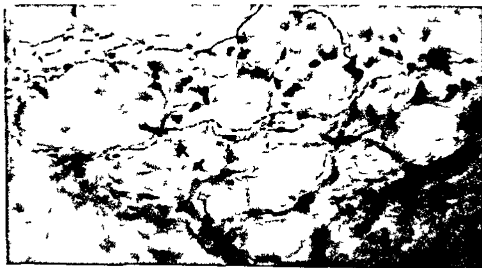


Fig 3 Norm l thyr d gl nd how ng bead d unmyelin ted r erv fib s coming into los r lat onsh p w th macrophag yncyt um a ound ac n (M thyl ne bl e × 00)

dermal coat and the inner set orientated parallel to the hair shaft the axoplasmic filaments lying within the vitreous layer of the follicle among the cells of the outer *root sheath* They come into close relationship with the Langerhans and clear cells in this situation

Buccal Mucosa

Using supravital methylene blue staining the epithelium of the mouth is observed to contain syncytial cells of the same type as those observed in the epidermis Often however they appear more rounded Nerve fibres pierce the epithelium in the same way as in the epidermis

Masson also demonstrated the presence of such cells (Langerhans and clear cells) in the buccal mucosa Boeke describes such cells as interstitial cells and Feyrter as Hellen Zellen in this situation

Ear

Beneath the epithelial cells and lying in the basement membrane of the nasal epithelium and its extensions lie typical Hellen Zellen (Feyrter 1953) (For descriptions of the vital staining of the ear see Fenton 1932) Macrophages are found in *the submucosa of the middle ear and Eustachian tube especially perivascularly* and in the connective tissue strands around the ossicles about the intrinsic muscles of the middle ear around the round or oval windows and in the membrana tympana especially at the peripheries They are also seen in the perichondrium of the Eustachian tube

In the developing ear macrophages occur as sub epithelial masses In aural polyps many macrophages are seen in the connective tissue especially around the blood vessels

Teeth

The enamel organ develops from the cells of the dental lamina a downgrowth of the primitive epithelium of the mouth into the underlying tissues This forms an invaginated inverted cup over the tooth papilla the dental germ The cells of the enamel organ are at first round or polyhedral and become differentiated into an outer cubical type the external enamel epithelium and an inner stratum of columnar cells or ameloblasts which form the enamel fibres and between these a network of stellate reticular cells The latter are separated by fluid which contains mucin and resembles a jelly This stellate reticulum is also present in the growth derived from the enamel organ the adamantinoma

For a review of the vital staining of the teeth see Lehner and Plenk (1936) In the enamel vital staining of certain cells has been reported frequently using numerous intravital dyes including methylene blue Like macrophages the cells are more numerous in the neighbourhood of caries or of copper amalgam fillings that is in areas of inflammation According to Zimmermann (19-7) the cells of the stellate reticulum of the enamel which are divided from one another by the adsorbed fluid stain in the same way as the myo epithelioid cells and the pericyte cells of the kidney glomeruli that is with chromic acid salts and thus appear to be macrophages As will be seen later the homologous cells of this stellate reticulum in adamantinomata

were found to take up both methylene and trypan blue dyes and are in fact macrophages The evidence points to the fact that the stellate reticulum is composed of macrophages

During the development of the teeth the formation of dentine depends on the activity of the odontoblasts Vital staining of macrophages in relation to the odontoblasts has been reported by many workers (see Lehner and Plenk)

The dental pulp consists of embryonic connective tissue with abundant gelatinous basophil ground substance and collagenous and reticulin fibres It contains numerous macrophages In the pulp a plexus of unmyelinated nerve fibres is found with endings described as lying between and in the odontoblasts (Kuntz 1953) These are the unmyelinated posterior nerve or trigeminal sensory root fibres

In the fully formed teeth odontoblasts and macrophages remain adjacent to dentine in the pulp cavity When dentine is denuded or the outside of the tooth irritated a production of new or secondary dentine may be observed on the wall of the pulp cavity and may fill it Odontoblasts play a role in the nutrition of dentine Dentine is sensitive to touch to cold acid containing food etc However only occasional nerve fibres penetrate the dentine and extend for short distances

In the teeth therefore the cells of the stellate reticulum of the enamel organ are macrophages In the dentine and pulp the odontoblasts are accompanied by macrophages near or in which are unmyelinated nerve fibres of the pulp

Thyroid Gland

Around the acini of the gland a syncytium of vitally staining stellate or ovoid cells is found lying in the basement membrane (Fig 37) The individual cells are in contact by means of their processes Similar vitally staining cells are found in the



Fig 37 Normal thyroid gland showing beaded unmyelinated nerve fibres coming into close relationship with macrophage syncytium around acini (Methylene blue × 00)

interacinar connective tissue spaces Unmyelinated nerve fibres with attached Schwann cells can be seen to pass in close relationship to the macrophage syncytium in the basement membrane in some sections Numerous unmyelinated fibres can be observed around the blood vessels in the interacinar spaces Using trypan blue the same vitally staining cells can be seen to be continuous with one another and with the pericytes of the capillaries surrounding the acini

Metachromatically staining macrophages have been described in the interstitial tissue of the thyroid gland by other workers (see Bargmann 1939) Williamson and Pearce (1926) described these cells as like Kupffer cells and as being large and stellate often with basophil and argyrophil granules *lying between the follicles and with the* processes of adjoining cells united to form a network They were in close relation ship with the basement membrane of the follicles and might indent their contour Their staining seemed to vary with the activity of the gland Cappell says macro phages are found around acini apparently between the blood capillaries and acinar cells being sometimes closely applied to the former and sometimes to the latter These cells give the typical staining reactions of and are otherwise identical with Hellen Zellen (Feyrter 1953)

Nerve fibres have been found in the arterial walls and also unmyelinated fibres *running freely among the follicles but no terminations in contact with follicular* cells have been described According to Nonidez (1933) most of the follicles are not in contact with nerve fibres of the interfollicular plexus

Kidney

The basement membrane of Bowman s capsule and of the tubules and the inter stitial tissue of the kidney are formed of layers of reticulin With vital methylene and trypan blue staining the dye becomes concentrated on these structures The capillaries are everywhere surrounded by macrophages (pericytes) and these continue *into the glomerular tuft as the visceral epithelial cells surrounding the capil* laries Similar macrophages lie in the basement membrane of Bowman s capsule The tubules are everywhere completely surrounded by rows of macrophages con tinuous by their processes and also continuous or perhaps identical with the pericytes of the capillaries (Figs 38 39) They lie in or below the basement membrane of the tubules Unmyelinated nerve fibres are found running between the tubules in close relationship to the vitally staining cells but not ending on *the tubule cells* (Fig 39) The nerve fibres can also be observed to encircle Bowman s capsule in close relationship to the macrophages lying in its basement membrane They can also be seen to lie in close relation to the pericytes of the afferent and efferent glomerular vessels and to pass into the glomeruli Collections of macrophages with nerve fibres in close relationship are found in the capsule of the organ These macrophages are continuous by their processes with those between the tubules while some of the nerve fibres also pass into the intertubular spaces Most of the nerve fibres however *enter the organ with the blood vessels at the hilum*

Cappell (1929) also found vital staining of cells in the glomerular tuft and of cells between the capillaries and tubule cells The latter were more numerous at the apex of the pyramids Von Möllendorff (1930) describes the occurrence of



FIG 38 Kidney showing macrophage sy n yt m su ro n ling tubules (Trypan Blue × 00)

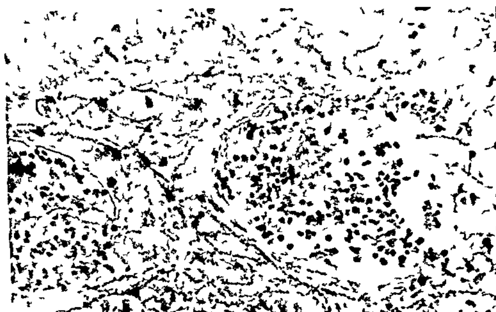


FIG 39 Kidney v tally sta ned w th m th ylene blue sh wing fin unmyel n ted nerve fib es ly ng betw n tub les an l n glomerul and mac oph ges tally ta n ng n gl merul and betw en tub l wh e th rve fib es ome nto rel t onsh p w th them (× 400)

numerous macrophages just beneath the capsule and also in relation to the basement membranes of the tubules and Bowman s capsule

Other workers have observed that nerve fibres enter the hilum accompanying the arteries Most of the fibres in the parenchyma are unmyelinated They run between the tubules and are said to be distributed solely in relation to blood vessels including juxta glomerular bodies and to glomeruli Unmistakable nerve fibres ending in relation to parenchyma cells have not been demonstrated (Kuntz)

The Pancreatic Islets

Using methylene blue and trypan blue stains the pancreatic islet cells are seen to be enclosed in and separated by macrophages and unmyelinated nerve fibres are in close proximity

Numerous macrophages surrounding the islet cells were described by Lubarsch (1921) and Cappell (1929) and Feyrter (1953) also records such cells but describes them as Hellen Zellen

The Interstitial Cells of the Testis

Using vital methylene blue and trypan blue staining the e yellow cells are found completely surrounded and separated by macrophages and numerous unmyelinated nerve fibres are in the vicinity

Vitally staining macrophages known as Zwischenzellen, have been described by *German workers as lying between the testicular tubules* They surround and intermingle with the yellow interstitial cells of Leydig (Takamori 1921 Cappell 1929 Schafer 1949)

Ovary

In the developing ovary the ova and surrounding follicle cells grow down as strands from the primitive germinal epithelium which covers the free surface of the ovary There is no basement membrane below this epithelium but a condensation of connective tissue called the tunica albuginea Within the substance of the ovary the ova are found surrounded by a number of follicle cells around which lies a basement membrane surrounded by the theca folliculi The latter consists of reticulin fibres and contains spindle shaped cells Lying in it are found vessels and nerves When the ovarian follicle ruptures the ovum is discharged and the follicle cells within the basement membrane become the lutein cells The spindle shaped cells of the theca with reticulin fibres now grow in between the follicular lutein cells as epithelioid cells containing lipid and cholesterol esters the so called theca lutein cells In atretic follicles the ovum and follicular cells show signs of degeneration and the cells of the theca interna proliferate and penetrate the basement membrane and enter the follicle They contain lipids The basement membrane becomes wavy and the cavity of the follicle filled with radially arranged strands of cells lying enclosed in reticulin

Vital methylene blue and trypan blue staining shows the presence of numerous macrophages throughout the ovarian connective tissue Unmyelinated fibres are found in enormous numbers and these appear to be applied to the surface of the

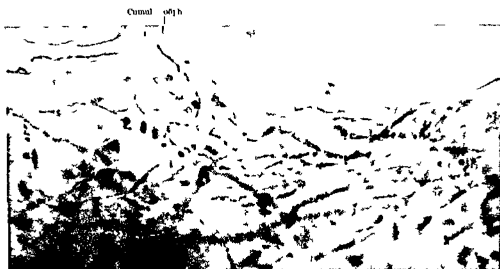


Fig 40 Wall of Graafian follicle showing macrophages and unmyelinated nerve fibres several of which pass into the cumulus oophorus (Methylene blue × 00)

Fig 41 Corpus luteum showing macrophages lying between lutein cells with unmyelinated nerve fibres in close relationship (Methylene blue × 700)

macrophages which almost seem to serve as Schwann cells to the fibres The ovarian follicles are surrounded by a syncytium of macrophages lying in the basement membrane in relation to which a collar of unmyelinated fibres can be observed (Fig 40) Unmyelinated fibres even pass into the cumulus oöphorus In corpora lutea stellate macrophages lying on reticulin fibres can be observed everywhere lying between the yellow luteal cells and forming a syncytium (Fig 41) The picture resembles the adrenal cortex, anterior pituitary etc *Fine unmyelinated fibres can be seen running between the luteal cells and in close apposition to the stellate cells* In atretic follicles bands of reticulin fibres can be observed running in various directions with numerous macrophages lying on them A few unmyelinated nerve fibres can also be seen A condensation of macrophages is present in the basement membrane of the germinal epithelium with numerous unmyelinated fibres below it

Vital staining of certain cells of the follicle and the corpus luteum was described by Aschoff (1924) and Keller (1942) Cappell (1929) says that throughout the stroma are vitally staining cells sometimes occurring in clumps and that vital staining occurs in both atretic and other follicles Kuntz states that most nerve fibres are unmyelinated The vascular bed is abnormally richly innervated Nerve fibres occur among interstitial secretory cells in the membrana granulosa of the follicles and in the *corpora lutea Nerves have also been found in the germinal epithelium* on the surface According to Kuntz it is doubtful if nerves penetrate the basement membrane of the epithelium of the follicles

Liver

All the parenchyma cells lying in the lobules are enclosed by reticulin fibres continuous with those of the connective tissue of the interlobular spaces and with that surrounding the bile capillaries and central veins of the lobule and continuous again with the reticulin of the capsule Great difficulty was experienced in staining this organ with methylene blue owing to the extreme rapidity with which the gland decolourizes the dye Eventually 1% solutions of dye were found effective Vital staining with both methylene blue and trypan blue stains the Kupffer cells lining the sinusoids and lying on the reticulin fibres interposed between the blood and parenchyma cells These are seen to be continuous by their processes to form a syncytium Similar vitally staining macrophages are found in the connective tissue of the interlobular spaces where they form the pericytes of the capillaries These are continuous by processes with the Kupffer cells at the peripheries of the lobules Macrophages are also found just outside the endothelium of the bile ducts and many lie under the capsule The latter are continuous by their processes with the Kupffer cells in the lobules immediately beneath the capsule Unmyelinated nerve fibres can be seen lying in relation to the blood vessels (arterioles and capillaries) and bile ducts in the interlobular spaces where they are in relation to the macrophages In the capsule also are found unmyelinated nerve fibres which lie in very close relationship to the macrophages Many are also seen perivascularly No nerve fibres were found penetrating into the lobules

Goldmann (1912) Cappell (1929) and many others describe macrophages occurring around the liver parenchyma cells in the periportal tissue and about the bile

ducts In addition the so called Stern Zellen are found floating in the blood of the sinusoids attached to the walls by one or two larger processes They may desquamate into the blood especially if the staining is intense (Cappell 1929) With intense staining the cells may break off into the lumen of the blood vessels or pass into the periportal connective tissue the central vein or beneath the liver capsule where collections are found *Riegele (1932) and other workers have shown that in the liver* spleen lymph tissue adrenal cortex anterior pituitary etc the reticular cells form a syncytium Riegele (1932) using silver stains claimed that in the liver spleen and adrenal cortex the axis cylinders of the terminal nerve network were intra plasmal and lay indeed in the syncytium of the reticulo endothelial cells The latter was so constructed that it formed the sheath of the axis cylinders that is it corres ponded to the replaced Schwann cells In the liver and adrenal cortex the capillary walls were formed of the reticulo endothelial syncytium The axis cylinders of the nerve *terminal* networks in the liver and adrenal cortex lay al o *in the wall of the* capillary network In the pulp of the splenic reticulum axis cylinders also coursed in the cytoplasm of the littoral cells I have not been able to confirm this in any of the e organs Alexander (1940) likewi e could find no nerve fibres running into or ending against the parenchyma cells or macrophages It seems possible that the fibres ob erved by Riegele were in fact reticulin fibres

Adrenal Gland

The cortex of the organ is composed of columns of cells lying on a reticulin framework dividing the cells from the blood in the sinusoids and continuous with that in the capsule and in the medulla of the gland The chromaffin cells of the medulla are likewise enclosed in reticulin fibres continuous with those of the cortex and also dividing the cells from the blood in the capillaries

Repeated attempts were made to stain the gland satisfactorily Methylene blue solutions containing 0 06% of the dye were necessary After infiltration the tissue must be immediately plunged into the fixative because decolourization is very rapid By this means the sinusoids can be observed to be lined by a vitally stained syn cytium of branching stellate cells (littoral cells) and the cells of the medulla are simi larly surrounded by these cells The medulla also contains numerous smaller vitally staining cells and larger blood vessels with the typical distribution of vitally staining cells in their walls Running in the capsule are found many fine nerve fibres both unmyelinated and myelinated Unmyelinated fibres run radially through the cortex in close relationship to the fixed macrophage cells lining the sinusoids (Fig 42) They continue into the medulla Other unmyelinated nerve fibres pass into the trabeculæ of the cortex and directly into the medulla where they appear in close relationship to the macrophages They do not appear to end on any medullary cells however Some ganglion cells are seen in the medulla

Many workers have described a similar reticulo endothelial structure of the adrenal gland including Cappell (1929) He also reports the presence of vitally staining cells throughout the medulla Riegele (1932) and others describe the littoral macro phages of the gland as forming a syncytium Feyrter (1953) records that these cells stain as Hellen Zellen According to Kuntz (1953) nerves enter the gland via

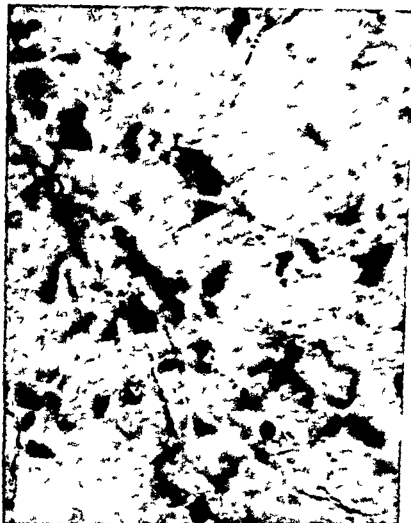


FIG 4 Adrenal cortex Beaded unmyelinated nerve fibres coming into close relationship with macrophage syncytium (Methylene blue × 400)

the medulla and capsule Many of the latter sweep round the glomerular arches in the outer cortical zone and extend inwards in the interfascicular septa Some terminate in relation to the blood vessels in the medulla without making terminal connections in the cortex The chromaffin tissue in the medulla is innervated directly through preganglionic fibres The postganglionic fibres are distributed to the vascular bed Most recent investigators fail to support the idea that the cortical cells are directly innervated (Kuntz) My own observations agree with this view

The Pituitary Gland and Pineal Organ

I have not examined these tissues The structure of the anterior part of the pituitary however resembles that of the liver and adrenal cortex and consists of

cells enclosed in a reticulin framework which divides the parenchyma cells from the blood in the blood vessels or sinusoids According to Cappell (1929) in the anterior pituitary vital staining occurs in large stellate cells with coarse irregular granules These are found just outside the capillary walls or as cells lining the sinusoids and analogous to Kupffer cells The reticulin framework with the supporting vitally staining cells forms a complete wall for the sinusoids of the organ separating the gland cells from the blood Some macrophages filled with secretion break off and wander into the posterior pituitary (Feyrter 1953) so called pituicytes As in the anterior so in the posterior pituitary vital dyes can be seen in endothelial and perivascular cells so called glial cells

The structure of the pineal gland resembles that of the anterior pituitary (Maximov and Bloom 1952)

The capsules of these glands are well supplied with unmyelinated nerve fibres and in addition fibres permeate the glands Some of these are unmyelinated and ending around the vessels and pericytes Nerve fibres do not apparently enter the parenchyma cells of these organs (see Kuntz 1953) In the anterior pituitary are found nerve fibres derived partly from the sympathetic accompanying the blood vessels and partly from the pituitary stalk They are believed to terminate freely in the reticulin connective tissue (Maximov and Bloom 1952) where they will be in close relationship with the macrophage syncytium

Parathyroids

The structure of these glands resembles that of the liver in that the parenchyma cells lie on and are enclosed by a reticulin framework The reticulin is continuous with that of the connective tissue surrounding the glands On it is found a syncytium of stellate cells staining with methylene blue and trypan blue and continuous with the macrophages of the surrounding connective tissue The macrophages form the walls of the sinusoids of the organ and separate the parenchyma cells from the blood In the connective tissue capsule are found unmyelinated nerve fibres lying in close relationship to macrophages

These vitally staining cells correspond to those described by Williamson and Pearce (1926) Cappell (1929) also described the staining of perivascular macro phages between columns of glandular cells Schafer (1949) reports numerous *sinusoidal blood channels running between the columns of parenchyma cells and* coming into close relationship with the cells The endothelium of these sinusoids is deficient in many places and may contain pigment Feyrter (1953) describes these same cells as giving the typical staining of Hellen Zellen According to Kuntz (1953) the nerves related to the glands are derived from those associated with the vascular bed Some unmyelinated fibres diverge from the course of the blood vessels and appear to terminate in relation to the secretory cells I was unable to demonstrate such fibres

The syncytium and reticular structure of this organ thus resembles that of the anterior pituitary and other endocrine glands The syncytium is in close relation ship to nerve fibres and thus intervenes between the nerve fibres and parenchyma cells

Lymphoid Tissue

This tissue is composed of a capsule made up of fibroblasts collagen and reticulin fibres from which trabeculæ pass inwards towards the hilum dividing the organ into segments and becoming *continuous with the collagenous* connective tissue of the hilum Lying in these segments and divided from the capsule and trabeculæ by the lymphatic sinuses are the lymphatic nodules which extend towards the hilum as the medullary cords The whole of the tissue between the trabeculæ is built

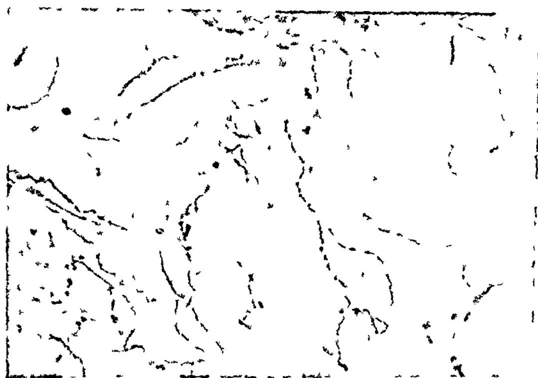


FIG 43 Lymphoid tissue from gut wall showing beaded unmyelinated nerve fibres passing into follicle (Methylene blue × [illegible]00)

on *a reticulin framework* enclosing the lymphocytes *developing from* the reticular cells and on which lies a syncytium of fixed macrophages The blood vessels and nerves of the lymphoid tissue are derived from two sources They pass in from the capsule and *the hilum by way* of the trabeculæ *The capillaries leave the* trabeculæ *to pass into* the lymphatic nodules forming a network in the periphery of the latter

Lymphoid tissue is *also very difficult to stain vitally* by methylene blue It is necessary to use *a solution of* 0 08% *strength Using this method numerous* macrophages with unmyelinated nerve fibres in close relationship can be demonstrated in the capsule (Fig 44) In the trabeculæ macrophages take up the dye and the

reticulin fibres also stain Unmyelinated fibres can be seen running in close relationship to the macrophages and capillaries (Fig 43) In the lymphatic nodules macrophages stain chiefly at the peripheries where they tend to form a chain encircling the germinal centre that is in the region of the capillaries surrounding those structures There may also be scattered vital staining of littoral cells of the syncytium in different parts of the germinal centre Sparse unmyelinated nerve fibres can be made out in close proximity to these vitally staining cells at the circumference of the lymphatic nodules If the macrophages are first stimulated by foreign proteins almost all the macrophages lying on the reticulin network can be made to stain vitally

In lymphoid tissue therefore there are found a capsule and trabeculæ containing macrophages and fibroblasts with blood vessels including capillaries



FIG 44 Capsule of lymph node showing macrophages in close relation to nerve fibre (Methylene blue × 300)

accompanied by unmyelinated nerve fibres The macrophages serve as pericytes for the capillaries Capillaries with their pericytes and accompanied by unmyelinated nerve fibres course round the edge of the lymphoid nodules The pericytes are syncytially related to the phagocytic cells on the reticulin of the germinal centres which enclose the developing lymphocytes

A similar arrangement of macrophages in the normal lymph node is described by Cappell In inflammatory states the number of vitally staining macrophages increases enormously and the reticulin framework is seen to be covered throughout by the macrophage syncytium

Spleen

This organ consists of a connective tissue capsule composed of fibroblasts collagen and reticulin fibres and a few unstriped muscle fibres From this trabeculæ of similar structure pass into the organ in various directions From the capsule and

Lymphoid Tissue

This tissue is composed of a capsule made up of fibroblasts collagen and reticulin fibres from which trabeculæ pass inwards towards the hilum dividing the organ into segments and becoming continuous with the collagenous connective tissue of the hilum Lying in these segments and divided from the capsule and trabeculæ by the lymphatic sinuses are the lymphatic nodules which extend towards the hilum as the medullary cords The whole of the tissue between the trabeculæ is built

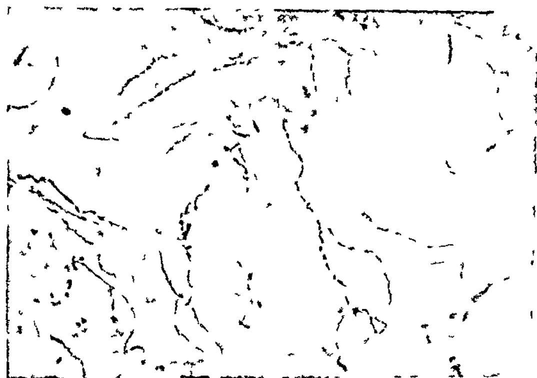


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replacing the macrophages of the adventitia of arterioles and to the edge of which nerve fibres reach by way of the vessels and (c) a set of macrophages lying on the reticulin of the red pulp and including the lining of the venous sinuses Free nerve endings seem to exist in the red pulp

The Bone Marrow

From the endosteum reticulin fibres pass inwards and form the reticulin frame work of the bone marrow on which lie numerous stellate cells taking up vital dyes and forming a syncytium In this reticulum are also found the precursors of the red blood cells and of the granulocytes The vascular endothelium of the inter sinusoidal capillaries lying in and surrounded by the reticulin framework also shows vital staining (see Cappell 1929 Maximov and Bloom 1932 and others) The reticulin meshwork and macrophage cell syncytium lie just outside the capillary blood vessels of the marrow The cells correspond to the pericytes (macrophages) in position

Fine myelinated and unmyelinated nerve fibres are found in the endosteum and the latter are present closely associated with the blood vessels in the marrow proper (Kuntz 1933) Some of these persist after sympathetic denervation Some fibres deviate from the blood vessel walls and end in delicate arborizing structures which have been thought to be receptor organs

Placenta and Amnion

The placental villi are covered by a syncytium of cells known as the syncytio trophoblast and beneath this lies a layer of cells the cytotrophoblast (Langhans) cells The latter lie on a reticulin membrane The centre of the villi consists of loose mesenchyme in which lie capillaries and numerous macrophages (Hofbauer cells) some lying immediately beneath the cytotrophoblast layer as in epithelia The wall of the amniotic sac is of a similar structure Enormous numbers of the cells of the basal decidua of the maternal tissue are macrophages The fœtal and maternal circulation in the villi are divided by the endothelium of the fœtal capillaries sur rounded by macrophages the cytotrophoblast the syncytiotrophoblast layer numerous macrophages in the maternal decidua and the maternal capillary sinusoid walls of the decidua surrounded by pericytes This constitutes the so called endo theliochorional barrier of Grosser

When vital dyes are injected into a pregnant animal the placenta fœtal mem branes and amniotic fluid are vitally stained but not the fœtus (Burrows 1932) The dyes pass from the macrophages of the maternal decidua into the syncytio and cytotrophoblast cells which show well marked vital staining with acid and basic dyes for example orange G and methylene blue (Goldmann 1912 Singer and Wislocki 1948 Franceschini) Lipids and iron also pass into these cells from which they are then taken up by the fœtal macrophages (see Wislocki and Dempsey 1946 1948 Singer and Wislocki 1948) This is the structure through which all oxygen and chemical interchange takes place between the blood of the mother and fœtus In fact this reticulo endothelial structure controls the metabolism of the fœtus

trabeculæ reticulin fibres pass inwards in all directions to form the framework of the organ in both the red pulp and Malpighian corpuscles On this lie the littoral macrophages which form a syncytium throughout the spleen The blood vessels enter and leave the organ at the hilum Lying in the trabeculæ the arteries pass inwards and then quit these to pass into the red pulp At this point the adventitia with its macrophages is replaced by lymphoid tissue (Malpighian corpuscles) The arteries continue inwards and break up into arterioles and capillaries

Using supravital injections of methylene blue directly into the organ and without previous stimulation by foreign proteins it is necessary to use 0 08% methylene blue and immediately plunge the tissue into the fixative This results in vital staining of macrophages and unmyelinated nerve fibres in the capsule and trabeculæ *and in the arterial walls In the red pulp the staining of the littoral macrophages is* capricious and patchy and occurs especially around the vessels In the lymphoid tissue the vital staining occurs chiefly at the circumference (Fig 14) Unmyelinated *nerve fibres may be observed in the trabeculæ surrounding the vessels and accom*panying the arterioles into the red pulp where they may be found in relation to macrophages They do not appear to have any specific endings If egg albumin is first injected a marked increase in the number of vitally staining macrophages is observed

The spleen has been described frequently as consisting of a reticulin framework in which lies a macrophage syncytium As in other organs not all macrophages stain vitally at the same time and they are indeed difficult to stain in the spleen (Cappell 1929) Cappell describes numbers of macrophages around the margin of the Malpighian corpuscles as a layer of stellate cells Neither the reticulo endothelial cells of the centre of the Malpighian corpuscles nor the lining cells of the venous sinusoids take up the dye The variation in staining has been attributed by Cappell to the existence of vascular shunts This however cannot be the case as it occurs when dye is injected directly into the tissue It must be due to their inactive state as they can be stimulated to phagocytose by appropriate means

According to Maximov and Bloom (1952) finely myelinated and unmyelinated *nerves with accompanying macrophages penetrate the hilum ramify in the reticulin* and collagen capsule and trabeculæ and some accompany the blood vessels into the organ They supply the inside of the capsule and trabeculæ They can be followed to the white pulp and even along the arteries beyond this Some also accompany the veins Many branches penetrate into the red and white pulp but their endings were considered as not definitely established (Kuntz 1953 Maximov and Bloom 1952) Some are found in relation to contractile cells (macrophages) of the wall of the venous sinusoidal spaces (Utterback 1944 see also Riegele 1932) As already mentioned Riegele claimed to have found the termination of the axis cylinders of unmyelinated nerves embedded in the cytoplasm of the reticular cells of the spleen and corresponding with and replacing Schwann cells a finding I was unable to confirm

Thus there seem to be (a) a set of macrophages lying in the trabeculæ and capsule among the collagen and reticulin fibres and in relation to nerve fibres, (b) a set of macrophages lying on the reticulin of lymph nodes (Malpighian corpuscles)



Fig 46 Skin from a case of scleroderma supravitally stained with methylene blue without previous injection of foreign protein showing the relatively few macrophages staining in the l rmis and Langerhans cells in the epidermis (Compare with normal skin) (× 00) The dark transverse line is the needle track

Fig 47 Connect t u t k n f m th d pth of a t oph sore of th ole of the foot in n l an d case f tab d l up a tally st ned w th methyl ne blu Note th d ffu e t in ng of the superfi al necrot t u and compl t bsen e of n rv fibres and ma ro phag s n the living tissue de p to t (00) Compa ed with F g 17 suggests the att t on of m ophag s to unmyel n te l n rve fibre

Vital Staining in Certain Pathological Conditions

(*a*) In three subjects injections of methylene blue solutions without prior injection of foreign protein were made into the abnormally innervated skin segment near but not affected by *herpes zoster*. The findings in this site differed from those in the normal in that there was a marked increase in the number of cells of Langerhans and clear cells staining. In normal unstimulated epidermis these cells are seen in



Fig. 45. Skin in same dermatome as lesions of herpes zoster showing swelling of epidermis with a marked increase in number of staining macrophages. (Methylene blue ×100.) Compare with Figs. 10 and 13.

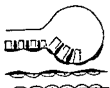
relatively small numbers but near herpetic skin the whole swollen epidermis shows large numbers of these cells throughout all the layers (Fig. 45).

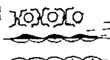
(*b*) In the thinned skin and subcutaneous tissues of areas affected by *scleroderma* the changes are the reverse of those seen in areas of herpes zoster. If vital staining is carried out without previous protein stimulation very few Langerhans and clear cells can be made out in the epidermis or macrophages in the underlying dermis (Fig. 46). If however foreign protein is injected prior to the staining considerably more macrophages can be demonstrated in the tissues. It seems that in areas of scleroderma the macrophages are not phagocytic unless strongly stimulated.



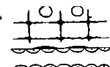












Diagr m ho ing h w th ll f th l body tiss lw divided f m th blood l tl pillaries by peri t (macro-

(c) The *anæsthetic tissues bordering on the painless trophic sores of the sole in three cases of tabes dorsalis* were examined Injection of vital dyes caused a diffuse staining of necrotic tissue but in the surrounding living tissue deep to this neither macrophages nor nerve fibres could be made out (Fig 47) The thinned epidermis in the vicinity of the ulcer similarly contained no demonstrable macrophages

Summary

To summarize the observations described above it seems that all tissues contain reticulo endothelial elements The capillaries are surrounded by macrophages in the form of pericytes in relation to which are found unmyelinated nerve fibres accompanied by Schwann cells Some of these are of posterior nerve root origin In the alveoli of the lungs the pericytes appear as the septal cells The larger blood vessels contain a syncytium of macrophages or adventitial cells lying in their walls and unmyelinated and finely myelinated nerve fibres (some of posterior root origin) in close relation to these cells

The peripheral nerves are surrounded by epi peri and endo neural cells which are fibroblast and macrophage in nature and into the epi peri neurium pass the unmyelinated nerve fibres of the nervi nervorum Macrophages appear to congregate around the unmyelinated nerve fibres in the connective tissue

Some of the cells lying between the fibres of collagenous connective tissue are not fibroblasts but are macrophage in nature and unmyelinated nerve fibres with free nerve endings are present near the cells which lie near true fibroblasts In the loose connective tissue unmyelinated nerve fibres seem to attract macrophages In the cornea between the corneal fibres are found similar macrophages with unmyelinated nerve fibres of trigeminal root origin coming into close relationship

The various tissues may be grouped according to the distribution of the reticulo endothelial cells in them (see diagram)

Type 1

Epithelial cells lie on a basement membrane in or on which lies a syncytium of macrophage cells which project between the epithelial cells Unmyelinated nerve fibres some of posterior nerve root origin form a plexus below and in the basement membrane and may pass up between the epithelial cells They come into close relationship with the macrophages This structure is seen in the respiratory transitional gastro intestinal and senile and immature uterine epithelia In the epidermis and its appendages there exists a syncytium of such reticulo endothelial cells lying between the other epidermal cells including the basal melanoblasts and unmyelinated nerve fibres of posterior nerve root origin accompanied by Schwann cells form a plexus below the basement membrane They afterwards penetrate to pass between the epidermal cells in close relationship to the macrophage syncytium A similar syncytium occurs in the epithelium of the buccal mucosa and in the enamel organ In the synovial membranes the synovial endothelial cells lie on a connective tissue containing macrophages forming a condensation immediately below the synovial cells Unmyelinated nerve fibres are in close relationship Some of these are of posterior nerve root origin In the perichondrium periosteum and endosteum the

nerve fibres are surrounded by macrophage cells in the form of microglia derived from the pericytes of the capillaries and unmyelinated fibres are everywhere found between the myelinated fibres in close relationship to the microglial cells

Type 5

Other organs consist of a reticulin framework enclosing the specific cells of the tissue On this framework lies a syncytium of reticulo endothelial cells dividing the parenchyma cells from the blood The framework conveys the blood capillaries with their pericytes into the organ The latter become continuous syncytially with the macrophages on the reticulin framework This structure is seen in fat lobules the liver adrenal cortex pituitary gland the parathyroids pineal body bone marrow corpus luteum lymphoid tissue the thymus and the spleen Often with the blood vessels unmyelinated nerve fibres some of posterior nerve root origin pass into the organ and may appear to end freely in the parenchyma These nerve fibres come into close relationship with the reticulo endothelial cells of the organs This is seen in fat lobules the adrenal cortex pituitary spleen lymphoid tissue bone marrow and corpus luteum In other organs the nerve fibres only appear to enter the capsule or the connective tissue surrounding the lobules of the organ in which lies a macrophage syncytium continuous with that lying on the reticulin framework of the interior of the organ or lobule This is seen in the parathyroids and the liver lobules In the bone marrow the endothelial cells of the blood sinusoids lying between the reticulin framework of the parenchyma also take up circulating substances

It can thus be seen that the blood is divided from epithelial and acinar gland cells by the reticulo endothelial cells on the basement membrane and between the acinar or epithelial cells from nerve fibres and ganglion cells by the neurilemma with its satellite and Schwann cell macrophages from muscle fibres by the reticulin sarco lemma and attached macrophages from cartilage and bone cells by the macrophages of the outer layers of the perichondrium and periosteum and endosteum and from fat cells endocrine gland cells pigment cells and hæmopoietic cells by a syncytium of macrophages in close relationship to which are found unmyelinated nerve fibres often of posterior nerve root origin

It has been shown that in the loose connective tissue of many organs unmyeli nated nerve fibres are surrounded by numerous macrophages and come into such close relationship with them that they appear to be closely applied to the surface of these cells or even to end in them It has been claimed by some for example by Stohr that unmyelinated nerve fibres end in the pericytes though I was unable to confirm this with the methods used Such nerve fibres certainly pass in close proximity to these cells In the cornea the corneal cells or corpuscles are macro phages and the nerve fibres entering the substantia propria of the cornea shed their Schwann cells and become applied to the corneal corpuscles which take their place The macrophages surrounding the odontoblasts also have unmyelinated nerve fibres in close relationship to or ending in them while Feyrter claims that the Hellen Zellen (macrophages) also have nerve fibres ending in relation to them Others believe that the interstitial cell syncytium and the Schwann cells are continuous Certain it is that there is a close relationship between the endings of unmyelinated

cartilage cells or osteoblast cells *on the surface of the tissue lie surrounded by* a condensation of connective tissue containing a syncytium of macrophages and fibroblasts with unmyelinated fibres in close relationship to the former

Type 2

In all exocrine glands the testis epididymis vesiculæ seminales and thyroid the acini and ducts lie on a basement membrane on or in which lies a syncytium of macrophages *forming* an open *network round the structure* Similar macrophages (? myoepithelial or basket cells) lie between the gland or duct cells Around this lies a plexus of unmyelinated nerve fibres in close relationship to the basement membrane and its contained macrophages The cells of the ovarian follicles are similarly surrounded by macrophages with unmyelinated nerve fibres situated in close proximity In the kidney the *basement membranes of Bowman's* capsule and of the tubules are in contact with a syncytium of macrophages derived from the pericytes Such pericytes accompany the capillaries into the glomeruli Unmyelinated nerve fibres run in *close association* with these pericytes and with the macrophage syncytium surrounding the tubules in the intertubular spaces

Type 3

In tissues consisting of small groups of cells producing specific secretions the cells are surrounded by and intermingle with macrophages Unmyelinated nerve fibres are found in close relationship to them This is seen around the odontoblasts the *cells of the pancreatic islets the yellow interstitial cells of Leydig in the testis the* adrenal medulla the pigment cells of the choroid and iris (and nævus cells in the dermis)

Type 4

Rows of macrophages with unmyelinated nerve fibres in close *relationship lie* on a reticulin membrane completely surrounding other cells *This* is seen in striped cardiac and plain muscle in which fibres are surrounded by rows of macrophage cells continuous by their processes and derived from pericytes They lie in close relationship to the reticulin sarcolemma of striped muscle which thus corresponds with the *basement membrane of epithelia In striped muscle these reticulo endothelial* cells are continuous with the corpuscles of the tendon many of which are macrophage in nature Unmyelinated nerve fibres some of posterior nerve root origin are found ending freely between the muscle fibres and in close relationship to the macrophage syncytium In unstriped muscle these cells are known as interstitial cells of Cajal The fibres of the peripheral and autonomic nerves are surrounded by the reticulin neurilemma on which lie the macrophage like Schwann cells and by numerous endo peri neural macrophages and fibroblasts

In the autonomic ganglia *and posterior nerve roots the ganglion cells are likewise* surrounded by a reticulin capsule on which lie the satellite or capsular macrophage like cells

Within the central nervous system and retina the nerve cells and myelinated

CHAPTER IV

THE VITAL STAINING UPTAKE OF CIRCULATING SUBSTANCES AND THE OCCURRENCE OF MACROPHAGES AND NERVE FIBRES IN MALIGNANT TISSUE AND BENIGN TUMOURS

VITAL and supravital injections were also made into and around malignant tissues using both methylene blue to stain nerve fibres and macrophages and also trypan blue Janus green B and Indian ink The injections were made into the centre of the tumour and also where it abutted on to normal tissue both with and without previous injection of foreign protein Numerous malignant tumours were investigated These included 23 examples of carcinoma of the breast 21 of carcinoma of the colon or rectum 16 of carcinoma of the stomach 23 of sarcoma of soft tissue or bone 6 of carcinoma of the kidney 8 of carcinoma of the cervix 33 of epithelioma of the skin or stratified epithelial mucosa 8 of carcinoma of the bronchus 6 of malignant melanoma of the skin and other tissues 9 of carcinoma of the bladder 2 of Wilms tumour 4 of malignant teratoma 2 of neuroblastoma 2 of osteoclastoma 2 of chorionepithelioma and 8 gliomata of various types Twenty six examples of rodent ulcer were also injected With sarcomata and very cellular carcinomata and melanomata no vital staining of nerve fibres or macrophages in the centre of *living* malignant sarcomatous melanomatous or cellular carcinomatous masses was obtainable (Figs 48–51) Methylene blue was rapidly and completely decolourized by living tumour tissue Other dyes diffusely but patchily stained the growths when injected directly into them especially areas of necrotic tumour cells Nearer the edge of the tumour rarely nerve bundles of the invaded tissue may be seen



FIG 48 Edge of carcinoma of breast abutting on to normal tissue vitally injected with trypan blue on left and Janus green B on right showing failure of uptake of dyes by malignant tissue

nerve fibres and macrophages in various guises interstitial cells or Hellen Zellen and that these cells appear interposed between nerve endings and effector cells or are associated with the naked nerve endings of unmyelinated posterior nerve root fibres

In normal tissues macrophages appear to congregate around unmyelinated nerve fibres When the latter degenerate as in cases of tabes dorsalis the macrophages tend to disappear from the denervated tissues

Fig 30 F lg of carc noma of uterus butt ng on to normal tissue showing collection of macrophages in v ous shapes around edge of growth and unmyel nated n rve fib e top p ng ho t at thi point (Methylene blu x 00)

Fig l F lg of a n ma of l rea t tally sta ned with tryp n blue h wing non stain g of n t ue and a llect on of mac oph ge at m rg n of tum ur t ue (x 00)

Without prior injection of foreign protein at the edge of each tumour there was often seen a zone of invaded normal tissue which took up the stain poorly. More often collections of functioning macrophages occurred at the edge of the tumour and in a few connective tissue septa penetrating it (Figs 48–51). Outside this zone macrophages and nerve fibres could be demonstrated normally. In acellular scirrhous growths there are found macrophages in the connective tissue around the malignant cells but none within the foci or columns of cells.

In the normal epidermis there are seen vitally staining clear and Langerhans



Fig 49 Subcutaneous metastases of carcinoma of breast vitally injected with trypan blue on left and Indian ink on right prior to removal from the body showing failure of uptake of dye or colloid by the growth (× 4). The darker spots in the tumour are the sites of intratumoral injections

cells. *At the site of development of carcinoma of the skin the epithelium with its* normal clear and Langerhans cells dips down into the corium and its vitally staining cells and nerve fibres gradually disappear (Fig 52). None can be found in the columns of malignant tissue below nor within the epithelial pearls though around these is found a condensation of macrophages.

In mice bearing spontaneous breast tumours stained vitally by intraperitoneal injections of trypan blue or isamine blue the findings were identical with those obtained in human material. The dye was concentrated in macrophages around the edge of the tumour and in connective tissue septa passing into the tumour but the tumour material was unstained.

When *pontamine sky blue was injected into human organs* bearing malignant tumours and the nodes bearing secondary deposits excised it was found that the

circulation in malignant tissue as it fails to occur even if the dyes or colloidal suspensions are injected directly into the substance of such tumours Furthermore it cannot be due to some abnormality of the chemical metabolic changes which transforms the methylene blue and other dyes into some colourless compound as there is no retention of vital dyes which do not undergo this change Necrotic tumour cells may give a diffuse staining with dyes *It must be concluded that there are no functioning macrophages in or innervation of malignant tissue* or that these cannot be demonstrated by supravital methylene blue staining Against the latter however is the fact that occasionally nerve fibres can be seen passing through the edge



FIG 53 Columns of cells of rodent ulcer showing surrounding macrophage syncytium (Methylene blue × 00)

of malignant tissue from normal to normal tissue surrounding the tumour and also that nerve fibres could not be demonstrated by silver stains This applies also to osteoclastomata and gliomata

The vital staining of *rodent ulcers* by methylene blue is difficult owing to rapid decolourization of the dye By repeated attempts the overall picture could be pieced together Methylene blue solutions of 0 06% strength are best It was found that the columns of basal cells lie on a reticulin membrane which stains fairly easily The cells of the growth do not stain vitally No Langerhans cells were demonstrable in the cell columns Lying on the basement membrane were found collections of macrophages often spread out to form a network individual cells being continuous by their processes (Fig 53) Occasionally very fine nerve fibres were demonstrable in relation to the macrophage cells but not penetrating the columns of basal cells Numerous macrophages and some nerve fibres could be seen in the stroma

tumour tissue in the lymph nodes failed to take up the dye while the normal lymphoid tissue around stained deeply

When pieces of the same malignant tissue which had failed to show any nerve fibres when stained with methylene blue were stained for nerve fibres by Holmes method they *again failed to show the existence of nerve fibres in the malignant tissue*

In the two examples of osteoclastomata the tumour tissue itself failed to take

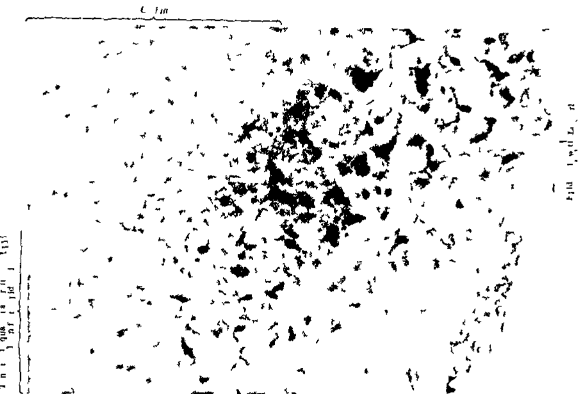


Fig 5? Tar epithel oma of sl in developing from epidermis On the left is a tongue of mal g nant tissue and on the right normal epiderm The macrophages (Langerhans cells) and nerve fibres within the normal epi lermis disappear in the malignant tissue (Metl ylene blue 200)

up any dyes or to show any nerve fibres in the same way as other malignant tissue The surrounding periosteum showed many macrophages and much reticulin The *two neuroblastomatous growths showed complete absence of any staining with vital* dyes like other malignant growths in contrast with the findings in ganglioneuromata None of the examples of gliomata of various kinds examined showed any vital staining though collections of microglial cells were found at the edge of the growth and microglial cells and unmyelinated fibres stained supra vitally within the sur rounding intact nervous tissue

Thus there seems to be no uptake of vital dyes or colloidal suspensions of Indian ink by macrophages in living malignant tissue This cannot be due to failure of the dye or colloids to reach the macrophage cells owing to some abnormality of the

circulation in malignant tissue as it fails to occur even if the dyes or colloidal suspensions are injected directly into the substance of such tumours Furthermore it cannot be due to some abnormality of the chemical metabolic changes which transforms the methylene blue and other dyes into some colourless compound as there is no retention of vital dyes which do not undergo this change Necrotic tumour cells may give a diffuse staining with dyes *It must be concluded that there are no functioning macrophages in or innervation of malignant tissue* or that these cannot be demonstrated by supravital methylene blue staining Against the latter however is the fact that occasionally nerve fibres can be seen passing through the edge



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The vital staining with acid dyes of malignant tissue in *animals* has been mentioned by Da Fano (1910) Anitschkow (1912) Goldmann (1912) Kiyono (1914) Weil (1916) Marsh and Simpson (1927) Ludford (1929 1931) Hess (1940) and Gersh and Catchpole (1949) Duran Reynals (1939) studied it in more detail and reported that in certain conditions tumours may appear by colouration to have taken up vital dyes in a highly selective fashion Brunschwig Schmitz and Clarke (1940) carried out a study of the accumulation of dyes in human tumours and reported a selective staining of tumour tissue in twenty out of thirty malignant growths with non staining of benign tumours and chronic inflammation However neither Duran Reynals nor Brunschwig *et al* describe the dye as being taken up by macrophages in tumour tissue The other observers agree that in malignant tissue including sarcomata skin carcinomata and ovarian carcinomata of the mouse there may be a collection of vitally staining macrophages around the edge of the tumour looking like a wall or capsule From the stained fibrous capsule diffusion of the dye into the normal tissue was sometimes seen Macrophages may also occur in connective tissue septa passing into the tumour from its edge Occasionally there have been described a few macrophages around degenerating areas of a tumour The same absence of macrophages was found in secondary tumours Dead tumour tissue or the boundary zones between normal tumour and necrotic centre were diffusely stained by vital dyes Central necrotic areas were not stained Tumour cells were absolutely free of dye

In attempts to outline the lymph drainage of human organs Weinberg and his co workers (Weinberg and Greany 1950 Weinberg and Morris 1953) at operation injected vital dyes especially 2% aqueous pontamine sky blue and direct sky blue into the normal parts of certain organs such as the stomach lung colon breast neck etc affected by carcinoma The dye colours the lymph channels and nodes a deep blue 10 to 15 minutes later They observed that metastases in the nodes did not take up the dye while the surviving lymph node tissue around stained deeply This corresponds with my own observations with these dyes

Tobin and Moore (1943) synthesized radio active dibrom derivatives of trypan blue and Evans blue containing Br^8 They gave intravenous injections and found that the uptake by malignant tumours was low as compared with other tissues except where necrosis was present and that the claims that the tumour tissue takes up more dye as judged by colour are erroneous and based on false appearances resulting from the whiteness of background of tumour tissue as compared with that of other organs

The evidence suggests that an essential feature of malignant tissue is the failure to abstract dyes particles and possibly physiological substances from the body fluids in the normal fashion

In view of my own inability to find nerve fibres in the centre of malignant tissue it is of interest to inquire as to previous work on this subject Willis (1952) considers at length the relationship of nerve fibres to malignant tumours and reviews the literature It is well recognized that intact nerve bundles or large nerves may be present within the substance of growths which have destroyed and replaced all other landmarks A number of workers have been struck by the extraordinary resistance

of nerve bundles and fibres to neoplastic attacks (see for example Ernst 1905). Since entire nerves frequently escape invasion by enveloping growths it might be anticipated that solitary nerve fibres are sometimes to be found within infiltrating growths. Much controversy has centered around the question of whether these fibres are included residues of pre existing nerves or whether they provide a genuine innervation of the tumour cells. Young (1897) using methylene blue and Goldmann (cited by Herzog 1928) using Weigert's stain for medullary sheaths found nerve fibres in a number of malignant tumours and concluded they were inclusions. Itchikawa and his co workers (1928) also found what they considered were uniform nerves and nerve endings in experimental cancers in rabbits and believed them to be an integral part of the tumours but Nakamoto (1926) failed to confirm their presence and concluded that tar cancers contained only damaged pre existing nerves. In tar painted mice Julius (1929 1930) could not find any nerves in the supervening carcinomas nor could Ludford (1930) and Lazzarini (1931). Meissel (1933) on the contrary claimed to have demonstrated regenerating fibres sprouting from damaged nerves in tar tumours and growing into the tumour tissue but did not suppose them to innervate the tumour cells. Herzog (1928) and Willis (1932) using Bielschowsky's stains or modifications of it found nerves present in a number of human malignant tumours. The former concluded that they were residues of pre existing nerves and that no nerves were present in circumscribed primary or metastatic growths which had not infiltrated and mingled with host tissues that in and around spontaneous tumours no proliferation of nerves was present that no nerve endings were present in tumour cells and that save for possible vasomotor effects tumours are independent of the nervous system. Willis found occasional nerve fibres present in the stroma and also among tumour cells but could not find any evidence that they were other than included residues. He pointed out that in metastatic carcinomas in the brain silver staining may yield pictures of residual nerve fibres extending into the tumours and assuming relationships with the tumour cells but found it impossible to conclude that these fibres innervated the tumour cells. On the contrary Martynow (1930) studied a series of squamous cell carcinomata and claimed to have found abundant newly formed nerve fibres ramifying amidst the tumour cells and even penetrating the cornified epithelial pearls. Oertel and his co workers (1928 1929 1931) claim to have demonstrated intimate connections of nerves with tumour tissue and even nerve endings in tumour cells. Shapiro and Warren (1949) thought that experimental tumours in the anterior chamber of the eye showed contraction of blood vessels on sympathetic stimulation and also that nerve fibres were present in the tumour. They concluded that functioning fibres had grown into the tumour around its blood vessels and might control the rate of its growth by controlling its vascular supply.

Against the claim that malignant tumours are innervated there are serious objections —

(1) Intact nerves large and small frequently persist deep within and closely surrounded by tumour tissue. It is inevitable that residual fibres should be included in the stroma of infiltrating growths and sometimes mingle intimately with tumour cells.

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Fig. 4 Fibroma edge as the growth abuts on to normal connective tissue on right showing beaded unmyelinated nerve fibre in the latter but not in the former The tumour edge marked by the dark staining pericytes of the capillary (Methylene Blue × 100)

Fig. 5 Uterine fibroid vitally stained with methylene blue showing presence of numerous macrophages between the plain muscle fibres and pericytes on the capillary walls Note the absence of nerve fibres (× 200)

(2) Silver impregnation methods are not specific for nerve fibres and may stain collagen and reticulin which may be mistaken for nerve fibres

(3) The appearances depicted by various workers and advanced as evidence of new growth of nerves or of the formation of nerve endings in tumours are unconvincing and many of them are typical of retraction bulbs and varicosities in degenerating nerve fibres

(4) Metastatic tumours at their inception clearly cannot possess a nerve supply yet they thrive as well or better than the parent growth

The evidence is strongly in favour of the fact that all genuine nerves found in tumours are inclusions and that nerves are not present in the centre of masses of cellular malignant tissue In acellular growths intersected by connective tissue septa obviously derived from the host nerve fibres may be found in the latter This conclusion appears to be favoured by so large a body of direct and circumstantial evidence that the doctrine of freedom of tumour cells from nervous control must be regarded as established (see Willis 1952)

Vital Staining of Benign Tumours

Using the methods described I have examined the vital staining of various benign tumours in a similar fashion to normal tissue

Fibromata Six examples of this tumour were examined These tumours consist of whorls of reticulin and collagen fibres lying between which are spindle shaped and branched fusiform cells Using vital stains even without foreign protein stimulation some of these are seen to take up the dye These macrophages are interspersed between the fibroblasts The capillaries of the growth are surrounded by pericytes continuous with the vitally staining cells of the tumour No nerve fibres are found in the growth At the edge of the tumour and in the capsule are condensations of similar macrophages Here there may also be observed unmyelinated fibres accompanied by Schwann cells and macrophages with which the similar cells of the tumour seem to be continuous (Fig 54) In two tumours areas of myxoma were present in the fibroma Here vitally staining cells lay in a mucinous matrix They were stellate rounded or fusiform and appeared to be macrophages

Neurofibromata Eight examples of this tumour were examined They consist of bundles of nerve fibres separated by a diffuse increase in all sheath structures consisting of reticulin and collagen Lying between the fibres are found cells of various shapes some of which take up vital dyes No unmyelinated nerve fibres are present in this tissue as they are in normal epi peri or endo neural tissue At the edge of the tumour the vitally staining cells of the tumour become continuous with the macrophages and the fibroblasts with similar cells of the endo peri neural tissues

Leiomyomata (fibroids) Nine examples of these tumours were examined They consist of whorls of unstriped muscle fibres surrounded by reticulin and collagen fibres and fibroblasts Alternating with the muscle fibres and fibroblasts are found numerous vitally staining macrophages with long processes They stain with both methylene blue and trypan blue The capillaries of the tumour are surrounded by



Fig Normal bloo l vessel on gl t widen ng o t into ang omatous essel sl ow ng n rve fibres ending at tl e jun t on In tl e walls of both normal an l abnormal v ssels ma ro pl ages are pres nt l ut in tl latter tl ey l ave a d sor le ly arrangement (M tl yl ne blue × 00)

Fi 58 Mu no ea of m x d pa ti l tum ur t lly tained w th trypan blu h wing the uptak f dye and the ent l ma roph ge n tur of many of th pleomorph cells in the m n (00)

vitally staining pericytes which are in continuous series with the vitally staining cells of the tumour No nerve fibres were found in the tumours (Fig 55)

Desmoid Tumour One example of this tumour was examined The findings were identical with those of fibromata

Lipomata Twenty four specimens of lipoma or of a mixed tumour containing lipomatous tissue were examined As in normal fat the cells of the lipoma are all surrounded by a reticulin framework on which numerous macrophages were present These were observed to be continuous with the pericytes of the capillaries Again



Fig 56 Lobule of lipoma vitally stained with methylene blue (× 320) Note absence of nerve fibres

as in normal fat connective tissue septa containing macrophages divide the lipoma into lobules In normal fat there can be found unmyelinated nerve fibres running in these septa and between fat cells but these could not be observed in lipomata (Fig 56) In fibrolipomata it was found that the macrophages surrounding the fat cells were accompanied by fibroblasts and the reticulin framework had increased in amount with the addition of collagen In angiolipomata the capillaries and smaller arteriolar vessels were dilated though the walls still contained macrophages which however in the arterioles were no longer elongated to surround the lumen as in normal arterioles but of various shapes The vasomotor nerves stopped short at the edge of the tumour

In the basement membrane lie the vitally staining macrophages surrounding the acini and on it other macrophage cells. In fibro adenomata of the breast the macrophage cells are still easily made out around and within the disturbed acini but the nerve fibres are not present (Fig 59). The acini and ducts are surrounded by excess of fibrous tissue with fibroblasts but devoid of nerve fibres. Some of the cells between



Fig 60. Adenoma of thyroid showing alveoli surrounded by macrophage syncytium but without nerve fibres (Methylene blue × 00)

the collagen fibres stain vitally. In cases of cystic change the cysts lined by glandular epithelium are similarly surrounded by macrophage cells and by nerve fibres in close relationship (Fig 20). In the cysts lying in the connective tissue and unlined by epithelium macrophages are often seen at the circumference of the cysts or in their mucinous contents but no nerve fibres are present.

Adenomata of the Thyroid Gland. Twelve examples of this tumour were examined. There was seen a change in the appearance of the vitally staining macrophages around the acini which now become elongated to look like fibroblasts. There is

Angiomata Five examples of capillary or cavernous angioma were examined In the walls of the dilated vessels were found plentiful reticulin on which lay numerous *irregularly shaped vitally* staining cells (Fig 57) No vasomotor nerves were found in relationship to the abnormal vessels They could however be observed stopping short at the point where the normal vessels entered the dilated vessels of the tumour At this point also the macrophages on the arteriole walls which showed a typical circumferential elongation in the normal vessel suddenly became irregular in shape *on reaching the vessels of the tumour*

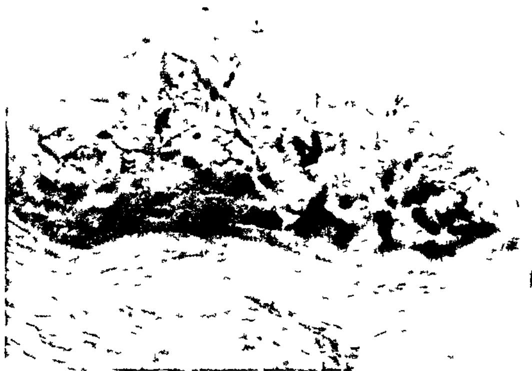


Fig 59 Fibro adenoma of breast Macrophage syncytium around compressed acinus surrounded by fibrous tissue containing no nerve fibres passing to the acinus (Methylene blue × 200)

Mixed Parotid Tumour Eight examples of these growths were examined In the *mucinous parts of the growths there* can be seen stellate and irregularly shaped cells taking up both methylene blue and trypan blue and the mucin also stains In the mucin are seen occasional deeply staining reticulin fibres The glandular epithelial cells do not stain Macrophages and reticulin are seen in *the capsule of the growth* where the macrophages of the mucinous areas merge with those of the capsule (Fig 58) No nerve fibres are present in the growth though they may be seen in the capsule

Fibro adenomata of Glands As shown above in all normal glands there is a plexus of unmyelinated nerve fibres with Schwann cells surrounding acini and ducts

vitally staining cells of various shapes lying between fibroblasts reticulin and collagen fibres the growths resembling fibromata No nerve fibres were found

Lymph Nodes from Cases of Lymphatic Leukæmia Six lymph nodes from cases of lymphatic leukæmia were injected with 0 08% methylene blue solution or 0 02% trypan blue There was a very rapid decolourization of the former dye and in order to obtain staining it was necessary to fix very rapidly The results showed a general increase in the number of staining macrophages in the node The staining was no longer chiefly confined round the peripheries of the germinal centres as in normal glands but the whole of the macrophages of the node often took up the dye

Congenital Malformations

Adamantinomata Two examples were examined The irregular columns and tongues of cells were found to lie in a matrix of connective tissue containing macrophages but no nerve fibres Among the cells forming the columns were found well marked vitally staining stellate cells continuous by their processes to form a network This is the stellate reticulum typical of the developing enamel organ

Pigmented Flat Nævi Seven examples of this birth mark exhibiting neither hyperkeratosis nor overgrowth of epidermal structures were examined It was found that the pigmented nævus cells (melanoblasts) in the corium were always in close relationship to pigment containing melanophores which took up methylene blue at the same time to give a greenish blue appearance In the epidermis the clear and Langerhans cells could be made out stuffed with melanin pigment and

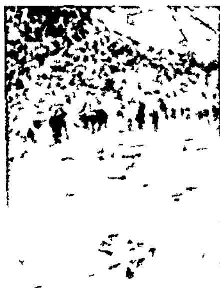


FIG 67 Raised hyperkeratotic melanoma vitally stained with methylene blue showing macrophages lying between basal cells and among pigmented cells in thickened epidermis A few nævus cells surrounded by vitally staining macrophages are present in dermis No nerve fibres are present in the lesion (× 100)

some increase in the reticulin and collagen around and between the acini No nerve fibres were found in the adenomata (Fig 60) Around the adenoma the interacinar connective tissue is condensed to form a capsule

Keratinizing Papillomata of the Senile Skin Twelve examples were examined In these there is observed a well marked staining of the Langerhans and clear cell layers of the thickened epidermis and of all the macrophages in the dermis (Fig 61) The capillaries are somewhat dilated and their pericytes stain No nerve fibres were found in the epidermis or upper layers of the dermis though they were present below this in the base of the growths



Fig 61 Hyperkeratinized papilloma of senile skin showing epidermis with Langerhans cell syncytium but absence of nerve fibre (Methylene blue 00)

Papillomata of the Rectum Three examples were obtained Again well marked vital staining of the macrophages of the basement membrane of the epithelium and between the epithelial cells and in the submucosa immediately beneath was found No nerve fibres could be seen in these layers though they are present in the centre of the connective tissue of the stalk in a normal manner

Feyrter (19_3) also found Hellen Zellen in the same situation in both nasal and aural polyps

Ganglioneuromata Two examples of this tumour were examined They showed among the ganglion cells and axons the presence of numerous vitally staining cells in the stroma among fibroblasts collagen and reticulin fibres and also vitally staining Schwann cells in relation to the coiled axons of the ganglion cells

Meningiomata Six examples were examined In all there were found numerous

tissues and appeared distributed in a normal fashion for example in periosteum and perichondrium in relation to blood vessels around gland acini and below epithelia Langerhans and clear cells were present in epidermis and hair follicles In two tumours nerve fibres with Schwann cells were found but the tissues did not contain the very fine network of unmyelinated nerve fibres found in normal tissues The nerve fibres were the axons of nerve cells present in the tumour and were not derived from the host tissues

The findings suggest that in fibromata neurofibromatous tissue leiomyomata desmoid tumours lipomata angiomata meningiomata papillomata of skin and mucous surfaces and hyperkeratotic pigmented nævi the over developed tissues contain no nerve fibres but macrophages are present as in normal tissues The same is true in adamantinomata where columns of cells resemble those of normal buccal mucosa in containing a syncytium of vitally staining cells In pigmented flat nævi the macrophages and nerve supply appear normal In fibro adenomata of glands there is an overgrowth of fibrous tissue devoid of nerve fibres surrounding the acini and the basement membrane of the acini on which lie macrophages and basket cells

In view of the above findings it is of interest to enquire into the results of previous workers In spite of prolonged searching I have been able to trace only one reference to the vital staining of benign tumours Brunschwig Schmitz and Clarke (1940) found no staining of such tumours Pigdon (1903) however mentions the presence of macrophages in the walls of angiomatous vessels

The innervation of benign tumours has been investigated by a number of workers Nakamoto (1926) found that the application of tar to the skin of the rabbit which causes the development of warty papillomata results in degeneration of the nerves of the epidermis and underlying dermis Neither Nakamoto nor Tsunoda (1927) could see nerves in malignant tissue but found them in benign growths Julius (1929) in the mouse found that papillomata induced by tar painting contained no nerves at first but if they persisted for long nerve fibres may grow into them The nerve fibres which originally ran to the surface became deflected backwards so that small areas of the epithelium were denervated Ludford (1930) found in the mouse that tar painting leading to papilloma formation in the skin destroyed the sub epithelial nerve plexus with its free nerve endings terminating among the epidermal cells but resulted in a considerable increase in the number of nerves in the dermis Around the margins of the tumour there was often found an intense proliferation of nerves believed to be due to regenerative activity following the destructive action of the tar upon the more superficial nerves Such an increase in the number of nerve fibres in the dermis was not found after long continued scarification In large pigmented hyperkeratotic raised nævi Soldan (1899) and Masson (1926) stressed the existence of local neurofibromatous changes in the adjacent tissue Willis (1948) describes the presence of nervous tissue in four fifths of all benign teratomata but the nervous tissue formed part of the tumour itself and was not derived from the host Nicholson (1937) however described the skin of a dermoid as being innervated from the host

Thus in regard to skin papillomata the majority of workers have reached the same conclusion as the author namely that such benign tumours are devoid of nerve

are even more pigmented than the rest of the epidermal cells They also stain with methylene blue The nerve supply of epidermis and dermis was normal and no trace of neurofibromatosis was found An interesting observation was that in several places uptake of melanin had occurred in the Schwann cells of the corium showing their macrophage nature

Hyperkeratotic (raised) Hairy Pigmented Nævi Five cases of pigmented nævi were examined in which the epidermis was enormously thickened and deeply pig



Fig 63 Edge of raised hyperkeratotic melanoma of skin on right with normal skin on left showing a leash of nerve fibres apparently deviated from the melanoma which contains no nerve fibre Same specimen as Fig 62 (Methylene blue × 100)

mented with or without excess of hair follicles and glands and with some degree of angiomatous formation of the vessels beneath In two examples numerous pig mented nævus cells were present in the corium In three others only a few such cells could be made out Large numbers of vitally staining enlarged and rounded cells were found throughout the thickened epidermis and in the basal layer corres ponding to the Langerhans and clear cells of the normal epidermis (Fig 62) Macro phages were present in the walls of the dilated vessels *No nerve fibres were made out in the epidermis or in the walls of dilated vessels* While no nerves could be made out in the nævus or beneath it *yet to one side of the growth* were one or more abnor mally large bundles of nerve fibres accompanied by Schwann cells and macrophages running up to the epidermis *outside* the nævoid area (Fig 63) This showed excessive development of the reticulin and collagen of its sheath

Benign Teratomata Four examples of benign teratomata were examined It was found that macrophages were present throughout the various constituent

The phenomena of inflammation differ from the mere collection of polymorph and lymphocyte cells locally in a tissue. Inflammation is a co-ordinated response of the tissues involving a nervous reflex and including local increased vital staining of macrophages, increased capillary permeability, diapedesis from the vessels, arteriolar vaso-dilatation, œdema, the collection of leucocytes and later healing and reparative processes. Inflammation is the result of *local cell damage* (see Burrows) and in normal tissues may occur in response to injury of any kind around an area of necrosis and



Fig 6. S[illegible]omata showing areas of hæmorrhage without inflammatory reaction (H and E × 160)

cell degeneration or in the region of hæmorrhage. X-irradiation of a tissue also produces a similar change. Since neither macrophages nor nerve fibres appear to exist in malignant tissue, it is of interest to enquire as to whether inflammation occurs in the substance of such tissue in response to those influences which give rise to inflammation in normal tissue, including trauma, chemical irritation, bacterial infection, necrosis and hæmorrhage. The author found that if the highly irritant substance mustard oil is dabbed on to a fungating sarcoma or cellular carcinoma of the breast it does not lead to inflammation and causes no obvious change in vascularity or temperature. When the irritated malignant tissue was removed twenty-four hours later it showed no inflammatory changes but only necrosis. In addition sections of many different types of malignant tumour which had either ulcerated to

supply in the area of overgrowth of epidermis and have remarked on the abnormality in the innervation of raised pigmented nævi

Clinical Observations on the Existence of Nerve Fibres in Malignant Tissue and Benign Tumours and on the Response of Such Tumours to Trauma Infection *Hæmorrhage and Necrosis*

Clinically sarcomata malignant melanomata and cellular carcinomatous tumour tissue appears to possess no pain sensibility This observation can easily be verified on fungating malignant tumours The author has frequently applied painful stimuli *such as pin prick pressure and pinching and even removing tissue for biopsy with*out using an anæsthetic and without any sort of pain being appreciated in such tissue Again when applied to the skin or mucosæ or exposed normal deeper somatic tissues, mustard oil produces intense redness tingling and pain, but is quite unappreciated when applied to a fungating sarcoma or cellular carcinoma unless absorption occurs when the normally induced responses including pain are observed in the tissues surrounding the tumour but not in the tumour itself

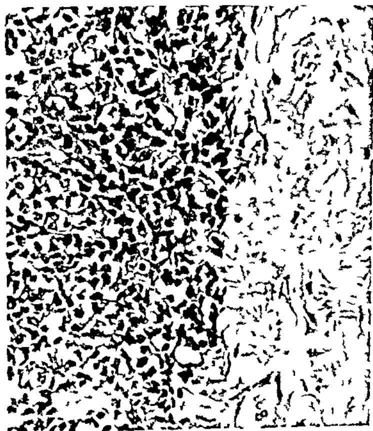


FIG 64 Sarcoma showing absence of inflammatory reaction to necrosis on right (H and E × 30)

Fig 6 Malignant melanoma showing area of necrosis on right sharply demarcated from the rest of the tumour without inflammatory response at the edge (H and E × 150)



Fig 68 Ulcerated very cellular carcinoma of breast showing absence of inflammatory reaction to infection (H and E × 00)

the surface or into the alimentary tract and thus were exposed to trauma or infection or which contained areas of hæmorrhage or necrosis in the *substance* of the tumour were examined microscopically for evidence of inflammatory response It is necessary to distinguish (a) sarcomata (b) melanomata (c) very cellular carcinomatous growths in which large columns or sheets of tumour cells occur in close apposition to one another without bands of normal connective tissue lying between the cells and (d) carcinomatous growths in which the tumour cells are sparse and



Fig 66 Ulcerating malignant melanoma showing no inflammatory reaction to infection (H and E × 150)

surrounded by the normal connective tissue of the invaded organ such as a scirrhous carcinoma or in which tumour tissue is intersected by bands of normal connective tissue In spindle round cell or osteogenic sarcomata or in malignant melanomata surface ulceration with infection or hæmorrhage or necrosis within the tumour produce no evidence whatsoever of any inflammatory reaction on the part of the intact tumour tissue (Figs 64–68) Trauma and infection lead to necrosis of the tumour tissue only There is no attempt at repair as in normal tissue In very cellular carcinomatous growths in which large areas of carcinoma cells are found with little stroma again no evidence of any reaction on the part of the tumour to trauma infection necrosis or hæmorrhage is found (Fig 68) Trauma and infection likewise cause necrosis without evidence of repair In scirrhous carcinomata or carcinomata with large amounts of host stroma infection leads to evidence of inflammatory

eight seconds in the symmetrical side on the normal limb The capillary pulse was almost absent in the nævus but normal in the corresponding area of the normal limb The blood vessels in the nævus in appropriate conditions appeared to dilate more rapidly and to a greater extent than in normal areas This suggests that the disturbed vasomotor reactions are due to lack of nervous vasoconstrictor tone

When ulcerated or traumatized benign tumours exhibit not only inflammatory reactions but are in fact more liable to such reactions than normal tissue These reactions are exaggerated and the tissues then have a tendency to mucoid degeneration Ulcerated fibromata exhibit typical inflammatory changes (Fig 69) Lipomata likewise tend to show inflammatory changes and areas of polymorph infiltration œdema and vasodilatation are frequently described between the fat cells When washed with soap which has no visible effect on normal skin I have repeatedly observed that hyperkeratotic angiomata become inflamed and show eczematous or psoriatic lesions The tissues of a deeper situated angioma may also show inflammatory responses On removal papillomata histologically often exhibit areas of inflammation Uterine fibroids are well known to have a tendency to inflammation and even pus formation (Ewing 1940 Willis 1948) in the absence of any organisms Ganglioneuromata frequently show collections of leucocytes (Willis) The stroma of a rodent ulcer also shows normal inflammatory changes Adenomata of the thyroid likewise are well known to exhibit areas of inflammatory change

To summarize clinical experimental and histological evidence appears to show that no nerves are present in either malignant or benign tumour tissue In flat melanomata they appear to be present in a normal manner In cellular malignant tissue functioning macrophages are not present though they are often collected around the edge of such tumours Such tissue shows none of the features of inflammatory response to agencies which excite these in normal tissues In benign tumours macrophages are present and there is an excessive tendency to and exaggeration of the changes constituting inflammation in response to noxa

Observations on the Triple Response in Pathological Conditions

When almost any agent whether chemical physical or radiation acts mildly on the skin it produces the triple response of Lewis (1927) This includes capillary vasodilatation with increased permeability and filtration of plasma contents into the affected areas There is also a local arteriolar vasodilatation dependent on the integrity of the posterior nerve root fibres evidently the C fibres of the skin At the same time histamine or H substance is liberated More prolonged action of the noxa causes inflammation The triple response is only an abbreviated or fractionated form of the phenomena of inflammation

The writer carried out a number of experiments on eliciting the triple response of the skin in different pathological conditions This was done by dragging the point of a needle along the skin with varying degrees of pressure In normal subjects such a procedure even with slight pressure causes a triple response which lasts five to ten minutes The same experiment was carried out in the anæsthetic skin immediately surrounding a painless *trophic sore* of the foot in three advanced cases of tabes dorsalis and in a similar situation in a case of complete traumatic section of the

response in the connective tissue stroma of the invaded organ *dividing the small* collections of tumour cells from one another The same is observed in the connective tissue between the columns of cells constituting a rodent ulcer It seems that tumour tissue as opposed to normal tissue does not exhibit the phenomena of inflammation in response to injury

Turning to benign tumours it is easy to confirm that the application of stimuli painful in normal tissues such as pinching or pricking to papillomata of the skin or hyperkeratotic (raised) pigmented hairy nævi (in which pulling on the hairs is often



FIG 69 Ulcerated fibroma showing violent inflammatory reaction in growth at top of section (H and E × 150)

not felt) is completely unappreciated Clinically the vessels of angiomata or the angiomatous element in other tumours show certain abnormal reactions Changes in the circulation affect both their size and colour In this respect Laignel Lavastine and Tinel (1920) and also Mativa (1925) describe two cases of extensive port wine nævus involving the neck side of the thorax and upper limb on one side In Laignel Lavastine and Tinel s patient on lifting the arms the nævus emptied of blood and the skin became almost normal in colour while the skin on the opposite arm blanched When the arms were now hung by the side the nævus attained its full port wine colour in five to eight seconds while the skin on the normal side did not do so for fifteen to twenty seconds Pressure on normal skin and on the nævus caused a white spot which disappeared in three to four seconds in the nævoid area and in seven to

PART II

THE ROLE OF MACROPHAGES IN NORMAL TISSUES

CHAPTFR V

THE CONTROL OF FEEDING AND EXCRETION OF OTHER BODY CELLS BY THE CELLS OF THE RETICULO ENDOTHELIAL SYSTEM

We have shown that all the cells of the multicellular vertebrate body lie in contact with and are surrounded by reticulin with attendant reticulo endothelial cells The reticulin of epithelia and glandular tissue is observable as the basement membranes in which the epithelial cells are embedded that of muscle cells as the sarcolemma that of peripheral nerve cells as the neurilemma and capsular membrane and that of cartilage and bone cells as the perichondrium and periosteum Each membrane has attendant macrophage cells respectively interstitial and basket cells myocytes Schwann and satellite cells perichondrial and periosteal macrophages The active cells of the appropriate tissues are further divided from the blood in the capillaries by the capillary wall with its attendant pericytes (macrophages) The acini of glands and their ducts are surrounded by connective tissue which brings the blood capillaries with their pericytes (macrophages) and nerve fibres accompanied by Schwann cells into close relationship with the basement membrane and its attendant macrophages (? myo epithelial or basket cells) The gland epithelial cells are divided from their foodstuffs in the blood by the capillary wall and its pericytes the interstitial and basket cells on the basement membrane Through these macrophage reticulin structures all nutrient hormonal and neurohormonal substances reach the acinar and duct cells from the blood and tissue fluids The same relationships are seen in epithelia and in muscle where the basement membrane or sarcolemma with attendant macrophages divides the cells from the capillaries surrounded by pericytes Cartilage cells draw their nutrient substances from the blood by way of the perichondrium formed of reticulin on which lies a macrophage syncytium a structure essential for the health of the cartilage The periosteum and endosteum serve a similar function in bone and are likewise essential for its continued existence Fibroblasts everywhere as in the nerve sheaths tendon and collagenous tissue are normally accompanied by attendant macrophages The reticulin neurilemmal and ganglion cell sheath and the satellite and Schwann cell macrophages divide the peripheral and autonomic ganglion cells and their axons from the blood in the capillaries surrounded by their pericytes In the central nervous system the microglial cells lying on the glial fibres serve a function corresponding to that of satellite and Schwann cells and the macrophages on basement membranes In the hæmopoietic tissues fat spleen liver pineal parathyroid anterior pituitary etc the parenchyma

sciatic nerve It was found that however hard the point of the needle was applied it was impossible to obtain any evidence of the triple response in these areas though the response could be readily obtained in areas where pain sensibility was normal

In a case of *scleroderma* en bande ' affecting the trunk the same procedure was carried out with the point of the pin being dragged across from normal to sclerodermatous skin and then again to normal While a brisk response was obtainable in the normal skin it was markedly reduced or almost unobtainable in the sclerodermatous area The same was found in the skin affected by *alopecia areata* Failure of ' reactive hyperæmia to occur in such skin is a well recognized feature of this condition

In the anæsthetic skin of several recently healed cases of ophthalmic herpes zoster the same procedure led to a violent response persisting for several hours and *in some cases this was followed by the appearance of herpetic blebs* The corresponding skin on the normal side gave a normal response

In two cases of *central pain* in the opposite half of the body following cerebral thrombosis of one hemisphere the skin on the painful side was hotter and redder than on the normal which is the usual finding A violent and widespread triple response was elicited on the painful side with great ease and merely by lightly touching the part

Below the level of a *transverse lesion of the cord* similar gross exaggeration of the triple response occurs In a case of fracture of the fifth dorsal vertebra with complete paraplegia of recent onset the effect of dragging the needle along the skin from above to below the level of the lesion was compared Above it produced the normal response *Below there was a gross exaggeration which persisted for hours* Kreibich (1927) records that in a case of cord compression by a meningeal tumour the effect of drawing a blunt object along the skin above and below the upper level of the sensory loss was different Above it caused an indistinct response below it resulted in a sharply demarcated response reaching in a minute to a breadth of 6 cm lasting half an hour and being produced by a lighter stimulus Irradiation of the meningeal tumour caused the difference in the responses above and below the lesion to disappear

It seems therefore that lesions of the central nervous system or peripheral nerves must in some way control the response of the tissues to noxious influence

Taylor, 1930). Thus it appears that the phagocytic activity of the pericytes may in part control the exit of substances from the plasma through the capillary walls and their access to body cells. The control of filtration through the renal glomerular capillaries would seem likewise to reside in part in the pericytes surrounding these structures.

Once out of the capillaries and in the tissue fluids, physiological or abnormal colloids are taken up by the *functioning* macrophages of the tissues lying in relation to glandular epithelial, fibroblast, cartilage, bone and muscle cells or nerve cells and fibres, and interposed between the blood in the capillaries and such cells. The adsorbed substances can be transferred from one part of the macrophage syncytium to another and thence to the other cells of the body tissues. By injection of acidic or basic vital dyes directly into the tissues of adult living animals, unless the cells are damaged, it is almost impossible to stain the parenchyma or epithelial cells of the tissues, as the dye is first taken up by the macrophages and even then the staining is very capricious (Bolles Lee, 1930). It seems that the *normal* method by which circulating substances are taken up by an organ from the tissue fluids is by being concentrated in non-specific fashion by the macrophages of the tissues interposed between the blood and parenchyma, muscle, epithelial and nerve cells, and subsequent transfer to the latter.

It was seen in Chapter I that a non-specific stimulus to, or activity of, a tissue results in increased phagocytic activity by its contained macrophages, and those macrophages previously not phagocytic may become so. As a result, both abnormal and physiological substances, including food substrates, present in the circulating fluids collect in increased amounts in and around the macrophages of the stimulated tissue and thus in the neighbourhood and at the disposal of the activated glandular, epithelial and parenchyma cells of the organ. It was also shown that in advanced cases of tabes the macrophages disappear in the denervated tissues. One of the features of this disease is the marked tissue wasting (tabes = wasting). Again in the tissues affected by scleroderma *functioning* macrophages are scarce. The tissues again exhibit marked wasting. It seems reasonable to conclude that the functioning macrophages of the various tissues serve to abstract and concentrate circulating food substrates, hormones, particles and cells from the body fluids and to pass them to the other cells of the body. They are local reservoirs of metabolic substrates. Tissue culture experiments have shown that the growth and division of normal cells is dependent on their food supply. Thus, by variations in their phagocytic activity, the macrophages may largely control the feeding and metabolic processes and so the growth of the other cells of the tissues. Diminution in such activity appears to lead to cell and tissue wasting and increase to cell growth and division. A stimulus of almost any kind increases the phagocytic activity of the macrophages of a tissue and so the foodstuffs brought to its cells, their metabolic processes and their rate of growth and division.

Such conclusions as to the trophic function of macrophages for other cells correspond to that described for these cells in tissue cultures by Carrel (1922). He showed that macrophages take up proteins from serum and the nitrogenous material of living or dead cells and transform them into food which they bring to

cells are enclosed in a reticulin framework on which lies the syncytium of reticulo endothelial cells and which divides *the parenchyma cells from the blood in the* capillaries with their surrounding pericytes The cells of the epidermis are also enclosed in a macrophage syncytium Fibroblasts the interstitial cells of the testis the odontoblasts and the cells of the islets of Langerhans are likewise always associated with and surrounded by macrophages and divided from the blood by capillary pericytes Thus all chemical exchange between blood and body cells must take place across this macrophage barrier The macrophage syncytium would appear to be situated in a position to control the *chemical exchange of other cells*

Lecithin and other phospholipids are lyophil amphoteric substances with an iso electric point about the same as the pH of the plasma They emulsify colloids Combinations of phospholipids such as lecithin with proteins fats and fatty acids carbohydrates inositol glycosides enzymes cholesterol digitonin and other sterols bile salts creatine and creatinine urea lactates quaternary ammonium bases basic and acidic dyes and metallic salts pyridine quinoline alkaloids such as quinine atropine caffeine strychnine etc and other substances readily form as complexes (Wittcoff 1951 Deuel 1955) It appears that many physiological substances such as fats carbohydrates proteins sterols etc are transported in the blood and tissue fluids as such phospholipid complexes Furthermore many abnormal sub *stances such as dyes metallic ions alkaloids etc when taken into the body also* become adsorbed on phospholipid protein complexes in the plasma Such complexes are hydrophilic and swell up to form gelatinous transparent masses

In Chapter I we have seen that when present in the blood and tissue fluids all substances whether physiological or abnormal basic or acidic colloidal or particulate cellular or phospholipid complexes with fats carbohydrates sterols proteins and hormones (usually sterols or proteins) become adsorbed on to and concentrated by the *active* macrophages of the tissues We have also seen that many substances taken up by the macrophages are passed on to the other body cells Many of the Kupffer cells of the liver (Sternzellen) are suspended in the circulating blood where they can take up substances from it and by *means of their syncytium can transfer them to parenchyma or gland cells* Substances absorbed from *the gut and passing* into the portal circulation are taken up by the Kupffer cells and passed to the liver parenchyma cells in this way Similarly toxic substances dyes or bacteria injected or otherwise introduced into a tissue such as the hand pass by way of lymph vessels to the draining lymphoid tissue and are strained off by the macrophages of the lymph nodes The same filtering off of bacteria dyes and phospholipids from the circulation is observed in the macrophages of the spleen The pericytes are situated in relation to the blood in a manner similar to the reticulo endothelial Kupffer cells and littoral macrophages of other organs When stimulated the pericytes (macrophages) around the capillaries must attract substances from the plasma through the capillary wall In fact the variations in calibre of the capillaries and outflow of plasma *and its contained food substances from the blood into the tissues* which accompany changes in pH anoxia noxious agents adrenaline and the application of various stimuli have been thought to result from a modification in the functional state of the pericytes (see Volterra 1925 Volterra and Schupfer 1934 Best and

flat moles the melanoblast or melanocyte cells are found in the underlying corium as the dopa positive so called naevus cells In or about these cell clusters are observed pigmented dopa negative macrophages (melanophores or melanophages) which have taken up the pigment By methylene blue staining the naevus cells are observed to be everywhere in close relationship to vitally staining macrophages the clear and Langerhans cells of the epidermis and the Schwann cells also take up the pigment and dye

It is well recognized that macrophages collect in relation to damaged cells and engulf and take up the débris of such cells They also take up damaged red and white blood corpuscles and dispose of their waste products In insects they take up urates and other substances and are concerned in their excretion (see Wigglesworth) The activity of tissue cells liberates metabolites such as histamine lactic acid and carbonic acid locally and these will likewise be taken up by all absorbing attendant macrophages of the active cells The metabolites dilate the capillaries (Schmidt 1930) and we have seen that such dilator actions are probably due to an initial effect on the pericytes (Volterra and Schupfer) *The macrophages appear to play an active part in removing the products of metabolism from neighbouring cells and so lowering their concentration both around and within other body cells* The perichondrial and periosteal macrophages would seem to subserve an essential role in removing the metabolites of the chondroblasts and bone cells as these membranes are the only pathways of excretion of the cells imprisoned in the solid structures Macrophages therefore would seem to play a part in delaying the fatigue of cells which is generally regarded as associated with the accumulation of metabolites Such deductions would explain why the activity of a tissue stimulates the local macrophages and increases vital staining and why the degree of vital staining in the reticulo-endothelial cells of a tissue is proportional to the metabolism of the tissue

That the removal of H_2CO_3 etc from active cells and their excretion is part of the function of macrophages appears to be shown by the large collection of macrophages in the alveoli of the lungs and the great increase in their number and passage into the alveolar spaces in conditions of heart failure when H_2CO_3 tends to accumulate in the tissues It might be supposed that these cells are in fact concerned in the transport of H_2CO_3 from active cells and its passage into the alveolar air Macklin (1954) concludes that there is a thin mucoid film containing acid mucopolysaccharides and lipids over the alveolar walls of the lungs probably the secretion of the macrophages He also considers the control of CO_2 and O_2 exchanges in lung alveoli is through these macrophages and their secretion He thinks they facilitate gaseous exchange

Hypertrophy (Hyperplasia) and Atrophy

Activity of a tissue results in a stimulation of the phagocytic activity of the local macrophages The latter will thus take up more food substrates from the tissue fluids and so the feeding of the cells of the organ will be increased and the cells grow This would seem to be the explanation of the so called *work hypertrophy* *Disuse atrophy* is also readily explained If the action of a mild stimulus to a tissue is prolonged it results in a stimulation of the growth of the tissues known as *hypertrophy*

fixed tissue cells The function of reticulo-endothelial cells might be described as opsonic that is preparing food for eating It corresponds to the function which Schwann cells are generally considered to subserve in relation to nerve fibres A nerve fibre is supplied by Schwann cells throughout its length It seems unable to function or even exist in the absence of such macrophage cells The Schwann cells serve a trophic function in relation to the axons of ganglion cells and the analogous capsule or satellite cells in relation to the ganglion cell bodies The same *trophic function of the macrophage syncytium is seen in the perichondrium and* periosteum in relation to the cartilage and bone cells which depend for their integrity on the covering membrane

The Uptake of Locally Applied or Injected Substances by the Macrophages of Tissues

The uptake of substances by the reticulo-endothelial cells occurs whether the substance is in the circulation or administered by injection or by application to the skin or epithelial surface This was shown to be true for vital dyes in Chapter II If thorotrast is introduced into the ducts of the mammary glands (C 1037) or pyrrol blue into those of the salivary gland (Staudacher Dalle o 1947) *or if Chinese ink is brought into contact with the tor* 1944) or fat with the lining of the small intestine (Leach on the basement membrane migrate to the epithelial s material and retraverse the epithelium and return to basement membrane This is known as histiocyt *draws attention to the evidence that drugs app* interstitial cells which appear to be macrop adsorbed by macrophages such as sterol or living organ from the body of an animal they This can be observed in the isolated living *atropine digitalis etc* are applied to the s adsorbed on to macrophages may later re

The Uptake and Removal of the endothelial Cells

Not only do the all absorbing loc lating substances and pass them to nerve or ganglion cells but also the these parenchyma cells must be taker This is well seen in the pituicytes of t endothelial cells full of the protein cells of the organ

Again the pigment producing cel occur in the basal layers of the epiderm has been the subject of much discus pass into the clear and Langerhans cells the pigment producing melanoblasts are or melanophores which take up the me

flat moles the melanoblast or melanocyte cells are found in the underlying corium as the dopa positive so called nævus cells In or about these cell clusters are observed pigmented dopa negative macrophages (melanophores or melanophages) which have taken up the pigment By methylene blue staining the nævus cells are observed to be everywhere in close relationship to vitally staining macrophages the clear and Langerhans cells of the epidermis and the Schwann cells also take up the pigment and dye

It is well recognized that macrophages collect in relation to damaged cells and engulf and take up the debris of such cells They also take up damaged red and white blood corpuscles and dispose of their waste products In insects they take up urates and other substances and are concerned in their excretion (see Wigglesworth) The activity of tissue cells liberates metabolites such as histamine lactic acid and carbonic acid locally and these will likewise be taken up by all absorbing attendant macrophages of the active cells The metabolites dilate the capillaries (Schmidt 1930) and we have seen that such dilator actions are probably due to an initial effect on the pericytes (Volterra and Schupfer) *The macrophages appear to play an active part in removing the products of metabolism from neighbouring cells and so lowering their concentration both around and within other body cells* The perichondrial and periosteal macrophages would seem to subserve an essential role in removing the metabolites of the chondroblasts and bone cells as these membranes are the only pathways of excretion of the cells imprisoned in the solid structures Macrophages therefore would seem to play a part in delaying the fatigue of cells which is generally regarded as associated with the accumulation of metabolites Such deductions would explain why the activity of a tissue stimulates the local macrophages and increases vital staining and why the degree of vital staining in the reticulo-endothelial cells of a tissue is proportional to the metabolism of the tissue

That the removal of H_2CO_3 etc from active cells and their excretion is part of the function of macrophages appears to be shown by the large collection of macrophages in the alveoli of the lungs and the great increase in their number and passage into the alveolar spaces in conditions of heart failure when H_2CO_3 tends to accumulate in the tissues It might be supposed that these cells are in fact concerned in the transport of H_2CO_3 from active cells and its passage into the alveolar air Macklin (1934) concludes that there is a thin mucoid film containing acid muco polysaccharides and lipids over the alveolar walls of the lungs probably the secretion of the macrophages He also considers the control of CO_2 and O_2 exchanges in lung alveoli is through these macrophages and their secretion He thinks they facilitate gaseous exchange

Hypertrophy (Hyperplasia) and Atrophy

Activity of a tissue results in a stimulation of the phagocytic activity of the local macrophages The latter will thus take up more food substrates from the tissue fluids and so the feeding of the cells of the organ will be increased and the cells grow This would seem to be the explanation of the so called *work hypertrophy* *Disuse atrophy* is also readily explained If the action of a mild stimulus to a tissue is prolonged it results in a stimulation of the growth of the tissues known as *hypertrophy*

(*hyperplasia*) Thus constant slight local stimulation of the skin by pressure causes an excessive growth of the skin including its appendages resulting in the formation of callosities with excessively large glands Similarly pressure over the periosteum leads to increased subperiosteal bone formation beneath the site Loeb (1908) discovered that during the development of the corpora lutea of the non pregnant guinea pig aseptic mechanical stimulation of the uterine mucosa by means of a glass bead or thread which causes a marked increase in the number of functioning macrophages resulted in the growth and differentiation of a small mass of decidual tissue (deciduoma) at the point of stimulation It has been shown that, when acting on the tissues of the body very many influences or stimuli result in an increase in phagocytic activity of the local reticulo endothelial cells as shown by an increase in their vital staining At the same time in the skin there is observable the triple response It seems probable therefore that a mild local stimulus including pressure causes hyperplasia because it increases local macrophage activity and thus the food supply of the growing cells An important feature of hyperplasia is that the over growth of tissues disappears when the stimulus causing it is removed In this it differs from benign tumour formation

Apart from a major or minor injury the tissues of the body are constantly stimu lated or minimally injured during normal living processes for example the epidermis alimentary tract mucosa and joint surfaces and normally slight reparative or regenerative processes are in progress in all tissues throughout life In other words regenerating influences seem to be constantly at work in all tissues and in view of the above deductions presumably result from local stimulation of macrophages by slight noxa or activity of tissues

Macrophages and the Phenomena of Inflammation

During the development of inflammation there occur capillary dilatation in creased permeability of their walls and escape of the plasma contents leading to œdema The vessel walls become sticky and leucocytes adhere to them and pass into the tissue spaces A burning pain is felt The vascular changes including hyperæmia capillary stasis and diapedesis are but part of the phenomena of inflam mation many of the features of which in experimental conditions may occur without arterial hyperæmia or capillary dilatation and the basic features of inflammation are found in avascular tissues such as the cornea Inflammation is followed by regenerative processes but where one ends and the other begins is impossible to define

The phenomena of inflammation have recently been considered by Robb Smith (1957) who points out that inflammation is not prevented by antihistamines so that release of histamine in the tissues by the noxious agent cannot be the initial distur bance in producing the phenomena as was once thought A whole range of poly peptides can be isolated from autolyzing tissue or tryptic digests of plasma proteins and produce some of the phenomena of inflammation when applied to tissues Many other vasodilator substances can be extracted from inflammatory lesions and plasma and liberation of these in the tissues has been claimed as the initial disturbance giving rise to inflammation These observations are however based on *in vitro*

experiments and have not proved convincing. Robb-Smith suggests that the primary disturbance is the degranulation of the mast cells which contain heparin, histamine and 5-hydroxytryptamine. These substances are liberated and may perhaps produce a change in the ground substance of connective tissue which becomes more fluid. Changes in the capillaries follow. There are however objections to this theory.

We have seen that the triple response is but an abbreviated or fractionated form of the phenomena of inflammation. The primary and essential feature leading to inflammatory change is damage to *living cells* (see Burrows, 1932). Inflammation is a response of the supporting tissues. All normal tissues contain macrophages, some of which are present as capillary pericytes. Noxious agents, chemicals and bacteria, which produce inflammation, are first taken up and act on the macrophages in the tissues. It has been mentioned already that dilatation of capillaries which accompanies inflammation with its increase in their permeability and the outflow of their plasma contents into the tissue spaces in response to a stimulus is dependent at least in part on altered function of the pericytes (Volterra and Schupfer) and there is every reason to suppose that the same mechanism operates in inflammation. Furthermore, we have seen that the triple response and inflammation only occur in tissues containing macrophages and may develop if these are the only cells present. This is observed normally in the substantia propria of the cornea, in which the only living cells present to be damaged are the corneal corpuscles or macrophages and mast cells are absent, and in the epidermis, where the only supporting tissues found are also macrophages. Again in the centre of malignant tumours, including sarcomata, melanomata and cellular carcinomata, no macrophages are present and factors which in normal tissues would lead to inflammation cause no such response. Also in anæsthetic areas of tabes, where nerve fibres are degenerated and few or no macrophages are found, noxious influences do not produce either the triple response or inflammation, but are followed by necrosis. It seems probable, therefore, that the *essential and initial step resulting in the phenomena of inflammation is disturbance of the macrophages, including the pericytes, of a tissue.*

In the cytoplasm of macrophages there are usually found lyophil phospholipid complexes, including protein compounds, and also histamine and antibodies, probably taken up from other cells which liberate them. The phospholipid complexes readily take up water and salts and swell. Normally vital staining by macrophages does not occur if the cytoplasm of the cells contains many phospholipid droplets. We have also seen that the action of any kind of noxious stimulus, including chemical, on a normal tissue, whether sufficient or not to give rise to obvious phenomena of inflammation, is accompanied by increased phagocytic activity by macrophages and increased vital staining and also by the liberation of histamine in the tissues. Thus any noxious stimulus to a tissue not only increases the phagocytic activity of macrophages and their uptake of circulating substances, but also appears to displace lyophil phospholipids and histamine from their cytoplasm into the tissue spaces. This must result in local imbibition of fluid and other substances with swelling of the tissues, stickiness of the vessel walls and attraction of leucocytes to the phospholipid complexes which seem to be the normal foodstuffs of most cells. The activated macrophages will now ingest the cell debris and waste products and also bring further foodstuffs

to the local cells and increase their metabolism. They concentrate antibodies and bring them to bear on the adsorbed antigenic substances. It should follow that the ease with which the triple response can be elicited is a test for the ease with which the macrophages can be made to liberate their contained phospholipids and histamine. Any kind of noxious stimulus to a tissue acts on its macrophages and leads to increase in all metabolic processes in the local cells with increased formation of their specific products, cell growth and division. When the stimulus or the response is excessive, the changes produced merge into those of inflammation, which thus occurs accidentally and not teleologically. This process would go on indefinitely unless inhibited by hormonal and nervous mechanisms, which will be considered shortly.

The "Sensory" Function of the Macrophage Syncytium

A small injury to the skin, mucosa or deep tissues is followed by an area of tenderness and increased sensitivity to other noxious and thermal stimuli spreading for a variable distance in all directions. It begins within a few seconds and lasts for hours or days according to the severity of the injury. The pain is diffuse, intense and prolonged, usually with a hot or cold element, and occurs both spontaneously or on stimulation, but the pain threshold is lowered only slightly. Lewis (1942) thought this phenomenon was due to stimulation of a specific set of nerves (nocifensor nerves) in the tissues, quite distinct from the pain fibres and stimulated directly by the injurious agent. These are not sympathetic, as the phenomenon is seen in tissues deprived of sympathetic innervation. The sensation is little affected by cocaine, but is abolished by ischæmia, whereas the reverse is true of other pain sensations. This suggests that pain nerve fibres are not directly responsible for the phenomenon.

Macrophages form a syncytium in the skin and other tissues and usually lie in relation to unmyelinated and specialized sensory nerve endings and end organs. Almost any noxious stimulus to a tissue stimulates the reticulo-endothelial cells and increases vital staining and phagocytosis over a variable area, depending on the severity of the stimulus. The effect of a stimulus to a part of the macrophage syncytium spreads to other parts. The increased phagocytic activity of the macrophages of a tissue must affect the metabolism of all nerve endings, including those subserving temperature and touch. While the stimulating effect of a noxa on the macrophage syncytium persists, the affected tissue would be expected to show an increased sensitivity to all other noxious and thermal stimuli. The reticulo-endothelial cells of tissues would seem to serve as a kind of sensory end organ. They may indeed constitute the nocifensor system of Lewis. Clark and Clark (1940) have also suggested that the pericytes, which are stimulated by various external, including chemical, influences, are chemo-receptors. Feyrter and his school likewise mooted the possibility of a sensory function of the Hellen Zellen (Buchner, 1944; Fröhlich, 1949).

General Effects of Noxa on the Body

Activity of a tissue leads to a local increase in the phagocytic activity of macrophages. This will tend to result in a localization of circulating substances or

organisms in the active tissue just as it does in inflamed tissue Thus can be explained the predilection of the virus of poliomyelitis for the active neurones in the cord and the chief effect of syphilis on the nervous system in brain workers and on the body tissues in manual workers

The introduction of toxins proteins or infective agents into the body has been seen in Chapter I to cause a general stimulation of macrophage activity throughout the body This of course increases the metabolic activity of all tissues and explains the tendency for non specific inflammatory changes in tissues for example arthritis nephritis myocarditis myositis and dermatitis after many intoxications and the increased rate of growth of hair nails bones etc seen in young subjects suffering from infections

The Effect of Cortisone on Macrophages

Cortisone it will be recalled is taken up by macrophages and when applied locally or by injection inhibits the inflammatory reaction of tissues to infection and toxic agents or to allergens It diminishes capillary permeability It inhibits the phagocytic activity of macrophages for typhoid bacilli and the normal restoration of phagocytic activity after depression by absorption of particulate matter (Biozzi *et al* 1957) It diminishes the pain shock and reflex phenomena of perforated peptic ulcer and may lead to rapid tiring and weakness of muscles Cortisone greatly inhibits antibody response (see Burnet 1957) Administration of cortisone often favours the spread of bacterial infections by depression of the resistance to organisms It inhibits the formation of fibres new vessels and granu lation tissue and suppresses the reaction to repair It may modify the invasive tendency of macrophages Inflammation following X rays is also inhibited by cortisone (Houghton Walter and Jones 1954) The fever and toxæmia accom panying inflammation are suppressed (Pagans 1952 Dunlop 1955) Thus cortisone would appear to act on macrophages and to diminish the phagocytic power and ease of displacement of adsorbed substances from the cytoplasm and so the inflammatory response In so doing the hormone must inhibit the local concentration of antibodies The secretion of cortisone after trauma or during infection and its take up by the disturbed macrophages thus tends to restore the status quo and is part of Selye s alarm reaction of the general adaptation syndrome

The Effect of Carcinogenic Agents on the Macrophages

The above observations as to the effect of noxa on the tissues apply equally to the carcinogenic hydrocarbons and the effect of these on the macrophage system has been investigated by a number of workers (see de Gaetani 1952) We have seen already that painting tar on to the skin of mice causes local increased vital staining of macrophages (Kreyberg 1927) When applied to the skin the effect of carcino genic hydrocarbons on the macrophages of tissues has been divided into four stages

(a) Marked increase in phagocytic activity of the reticulo endothelial system
(b) Commencing rarefaction of the macrophages
(c) The disappearance of the macrophages in areas of degeneration
(d) Areas of true neoplasm where no macrophages are present

X irradiation has been shown to have an identical effect (see Franceschini) It greatly inhibits antibody response (Burnet 1957) These agencies therefore stimulate and then depress the activity of and finally kill the macrophages according to the Dustin effect

The Uptake of Particulate Carcinogenic Agents by Macrophages

By analogy with other substances it would be expected that chemical or particulate carcinogenic agents applied to a tissue would be adsorbed on to the reticulo endothelial cells of the tissue Rous (1910) found that it was possible to transmit certain tumours in birds by means of cell free filtrates The agents are particulate and present in the blood of tumour bearing birds They may produce growths in *injured* or *inflamed* tissues that is where phagocytic activity of reticulo endothelial cells is increased The agent thus seems to be taken up by macrophages like other particles Similar observations have been made in cases of virus induced warts in man in whom the growths may develop in operation scars or sites of pressure (Köbner phenomenon)

The Migration of Macrophages

In certain conditions for example inflammation or local irritation portions of the macrophage syncytium of organs and the capillary pericytes round off migrate and form free macrophages In other conditions the nuclei of the cells divide but not the cytoplasm giving rise to giant cells (Metchnikoff 1893) Normally macrophages migrate towards injured and dying tissues In addition they are found collected around unmyelinated nerve fibres If a tissue is deprived of its unmyelinated nerve supply as seen in the anaesthetic areas of tabes or leprosy after a while macrophages are not found in the tissue in any numbers It may be that substances liberated from unmyelinated nerve fibres are chemotactic influences attracting the macrophages to their neighbourhood Invasion of normal tissues by macrophages may depend to some extent on antigen antibody like reactions Further reference to this will be made in Chapter VII

CHAPTER VI

THE NERVOUS CONTROL OF RETICULO ENDOTHELIAL CELL ACTIVITY

AUTONOMIC unmyelinated nerve fibres liberate neurohormones such as acetyl choline and (nor)adrenaline and possibly other substances The acetyl choline metabolism of nerves is only quantitatively more important at the synapses where the neuronal surface increases (see Fulton and Nachmansohn 1943) Acetyl choline has been shown to increase vital staining of macrophages Thus neuro hormones liberated in the vicinity of any part of an unmyelinated fibre would be expected to be taken up like any other substance by the neighbouring reticulo endothelial cell syncytium surrounding capillaries acini ducts etc and so their phagocytic activity and vital staining and local cell metabolism may be altered

It has been shown that in all situations where collections of macrophages are found unmyelinated nerve fibres often of posterior nerve root origin and their free nerve endings are present in close relationship This is seen around capillary blood vessels in relation to pericytes in the walls of the larger vessels in the endo perineural tissues in relation to tendon corpuscles in the basement membranes of epithelia and glandular acini the tubules of the kidney and the perichondrium periosteum and endosteum in the pulp of the teeth between the fibres of striped cardiac and plain muscle and between the myelinated nerve fibres and ganglion cells of the central nervous system around the cells of the cornea and in relation to the littoral cells of the adrenal cortex pituitary parathyroids corpus luteum lymphoid tissue thymus spleen and bone marrow and the macrophages between fat cells and also in relation to the peripheries of the syncytial network in the liver and in the macrophage cells around the interstitial cells of Leydig the islet cells of the pancreas etc In many of these situations the reticulo endothelial cells correspond to the interstitial cells generally recognized as interposed between nerve endings and effector cells and in others as Hellen Zellen or basket cells against which nerve fibres are stated to end Moreover in connective tissue it has been seen that macrophages tend to collect around bundles of unmyelinated nerve fibres It is thus reasonable to assume that the activity of macrophage cells is affected by nervous discharge

That nervous impulses do control the functioning of the macrophages and vital staining has been shown directly Thus if the cervical sympathetic nerve (which contains also posterior nerve root fibres) of the rabbit is cut on one side or one lingual or sciatic nerve of a dog is sectioned and a vital dye is injected intravenously then the organ on the denervated side becomes coloured before and to a greater extent than on the normal (Pogowicz 1885 and see Burrows 1932) Such nerve section may lead to temporary or permanent arterial and capillary vasodilatation and exudation from the vessels and œdema of the tissues

In certain circumstances for example in subjects of urticaria the triple response to a mild stimulus is more easily obtained and more lasting than normal a condition known as dermographism This occurs with certain diseases of the central nervous system such as meningitis and is then known as the *tache cérébrale* again suggesting the nervous system influences this response to a stimulus which is no more than the initial stage of inflammation

Various clinical observations show that nervous discharge in the unmyelinated fibres of the posterior nerve roots tends to inhibit the inflammatory response to a stimulus or injury and bring it to an end and thus presumably to inhibit or restore the phagocytic activity of macrophages to that before the application of the stimulus Before the evidence is presented it is necessary to consider the neurogenic origin of certain clinical conditions

The Course and Connections of the Unmyelinated C Fibres of the Posterior Spinal Nerve Roots and Trigeminal Sensory Nerve Root

In Chapter I we have described the origin of the unmyelinated C fibres of the posterior spinal and trigeminal nerve roots These fibres appear to arise or end as free nerve endings in all tissues in the blood vessel walls and in viscera and the fibres course in the lateral part of the posterior nerve roots to enter the ground bundles of the cord Connections are made by short relays in relation to the central grey matter with the hypothalamus by way of the medulla pons and midbrain Lesions of or near these pathways anywhere between the peripheral nerves and hypothalamus may give rise to disturbances of growth and nutrition of tissues and changes in their vascularity and response to noxious influences

Scleroderma as a Sequel to Lesions of the Nervous System

Scleroderma may occur at any age but usually appears in adults While its most obvious lesions affect the skin any tissue may be involved such as muscles bones joints intervertebral cartilages corpora mammæ mucosæ of the gastro intestinal tract bronchi trachea and larynx lungs myocardium thyroid kidney ovary bone marrow and eye (Bourne 1946–47 Groetz 1945 Hale and Schatzki 1944 Pugh Kvale and Margulies 1945 Bevans 1945 Prowse 1951 Medvei 1945 Lushbaugh Rubin and Rothman 1948 Lloyd and Tonkin 1949 Church and Ellis 1950) The cutaneous changes may have an irregular distribution though the clavicular region face neck and arms are most frequently affected They may however exhibit a *segmental* arrangement corresponding to one or several nerve roots (sclérodermie en bande) The zonal linear or unilateral distribution of this condition has been described frequently (for references see Ehrmann and Brunauer 1931) Scleroderma may be complicated by facial hemiatrophy in which the atrophic lesions occur in the distribution of the trigeminal nerve (Osborne 1922 Cassirer and Hirschfeld 1935) It has followed in the skin affected by herpes zoster It has complicated tabes dorsalis (Lortat Jacob and Legrain 1923 Guillain 1929 Cassirer and Hirschfeld 1935) In numerous cases of scleroderma degenerative changes have been found in and around the central grey matter of the cord or

medulla (see Ehrmann and Brunauer 1931) Leri (1926) and many others have described cases of scleroderma en bande in association with spina bifida occulta Scleroderma may accompany chorea Josephowitsch (quoted by Ehrmann and Brunauer) records the case of a nine year old girl who developed right sided Jacksonian epilepsy and six weeks after the onset of the attacks scleroderma appeared in the affected limb Scleroderma may also accompany post encephalitic Parkinsonism (Cassirer and Hirschfeld 1935)

Facial or General Hemiatrophy and Hemihypertrophy following Lesions of the Central Nervous System

Facial hemiatrophy is a special form of scleroderma and may occur in a patient suffering from zonal scleroderma (Osborne 1922 Cassirer and Hirschfeld 1935 and others) The manifestations of the disease are confined to the trigeminal *sensory* distribution In some cases a facial hemiatrophy is associated with an hemiatrophy of the rest of the body on the same or the opposite side (alternating hemiatrophy) Cases have followed trigeminal zoster (Trotter 1915) or complicated tabes Purves Stewart (1937) describes a case following an injury in the frontal region It may develop in cases of posterior fossa tumour or follow encephalitis lethargica (Hall 1924) It may follow a traumatic injury of the medulla (Leri 1926) Sometimes the lesions appear for no obvious reason shortly after puberty Clearly the manifestations of the disease result from a disturbance of nervous function

Facial hemihypertrophy is also confined to the sensory distribution of the trigeminal nerve It is distinguished from facial hemihyperplasia in that it begins after maturity It has occurred in cases of disease or damage to the medulla or trigeminal nerve for example in cases of syringomyelia after head injury or in cases of tabes dorsalis (Cassirer and Hirschfeld)

General hemiatrophy and hypertrophy are also acquired conditions Facial hemiatrophy may occur alone or in association with atrophy of the rest of the body on this side In other cases facial hemiatrophy on one side is found in combination with hemiatrophy of the tissues of the rest of the body on the opposite side This alternating type of hemiatrophy may be combined with a spastic paraplegia and may result from a lesion of the medulla for example syringobulbia or glioma A lesion of one side of the hypothalamus may be accompanied by general hemiatrophy (Cassirer and Hirschfeld 1935) Unilateral postencephalitic Parkinsonism with hemiatrophy of the affected side or a crossed hemiatrophy was described by Lange (1932) Hemihypertrophy of the body may also follow head injury encephalitis lethargica or syringobulbia Wartenberg (1945) cites cases of hemilateral disturbance of fat distribution observed after encephalitis lethargica cases in which facial hemiatrophy and lipodystrophy were associated with thalamic central pain and a peculiar affective state a case of unilateral post encephalitic Parkinsonism combined with hemiatrophy of the same side and cases of hemiatrophy accompanied on this side by hyperalgesia hyperaesthesia for cold and hyperhidrosis or by an Argyll Robertson pupil anisocoria acromegalic features exophthalmos extrapyramidal phenomena or by apoplexy and thalamic central pain Wartenberg considered the condition resulted from a lesion of the highest centres within the

brain probably the hypothalamus Cases of alternating hemiatrophy certainly point to a central origin

Alopecia Areata and Nervous Lesions

The role of nervous disturbance in producing this condition is generally recognized In some cases the patches of baldness appear in the area of supply of a definite nerve Alopecia areata may also occur in cases of tabes Leriche (1936) reported a case in which a spinal lesion at D7 level gave rise to persistent pain in this segment and two bald painful areas of alopecia areata appeared in the occipital region and the neck below it The pain was relieved by local anæsthetization and this was followed by regrowth of the hair

Herpes Zoster a *Phenomenon resulting from Lesions of Nervous Pathways*

The skin lesions of herpes zoster lie in the area of sensory supply of the posterior and trigeminal sensory nerve roots where the causative virus appears to prosper The author (Wyburn Mason 1957a) has found that not only the skin but all somatic tissues and the viscera supplied by the same sensory nerve roots exhibit lesions Lewis (1927) showed that herpetic and herpetiform eruptions occur as sequels to lesions of the sensory nerve tracts not only do they follow irritative lesions of the (root) ganglion but they are produced also by lesions of those tracts distal to the ganglia themselves The fibres concerned appear to be unmyelinated C fibres

Causalgia

This painful condition tends to follow minor lesions of the median or sciatic nerves and develops at various intervals after the injury The tissues supplied by the affected *sensory* nerves show many inflammatory changes and growth disturbances

" Central " Pain

Central pain may result from lesions in the neighbourhood of the thalamus and hypothalamus and of the central grey matter of the midbrain pons medulla or cord (Riddoch 1938a) It affects the tissues of the opposite half of the body which are the seat of a severe dull aching burning pain occurring spontaneously but also precipitated by stimuli of all kinds exteroceptive proprioceptive and visceral Often there is apparent analgesia of the affected part

Thus all the above conditions would appear to result from lesions of nervous pathways in the peripheral nerves or around the central grey matter of the spinal cord bulb or midbrain or of the hypothalamus

Evidence for the Inhibitory Nervous Control of the Phenomena of Inflammation

There is clinical evidence which suggests that normally nervous impulses inhibit the phenomena of inflammation

(1) In cases of herpes zoster typical inflammatory changes are found in the skin and other affected tissues As already shown the neighbouring epidermis shows increased numbers of vitally staining Langerhans and clear cells as compared with

normal The inflammatory changes are also found in the deeper parts of the corium in the hair roots and in the walls of the vessels which show swelling of the media and infiltration of the intima Subcutaneous fat may contain knots of inflammatory cells

The unmyelinated posterior nerve root fibres supply not only the skin but also the deep tissues and inflammatory changes may likewise involve the deep tissues supplied by the affected nerve roots (Wyburn Mason 1957a) Similar posterior nerve root unmyelinated afferent nerve fibres pass to the viscera The writer (Wyburn Mason 1957a) has shown that in cases of herpes zoster inflammatory lesions are found in the viscera supplied by the affected afferent nerve roots

We have seen above that the triple response is exaggerated in the rash free skin of the affected dermatome during an attack of herpes If a line is drawn with a finger nail on the skin immediately adjacent to the lesions of herpes zoster there is a gross exaggeration of the triple response (dermographism) and the site of pressure becomes pigmented for more than a month (Souques 1921 Thomas 1925) Furthermore I have observed that such stimuli may cause the appearance of zosteric vesicles in the stimulated area which was previously free of such lesions Thus in a woman of seventy one years a subject of herpes zoster confined to the area of supply of the supra orbital nerve on one side the application to the frontal region on this side of warm water easily tolerated over the normal skin resulted within a few hours in a violent inflammatory reaction and the whole of the skin supplied by the ophthalmic division of the trigeminal nerve including the eyelids became acutely inflamed Eight weeks later after healing had occurred there remained almost complete analgesia in the ophthalmic division of the trigeminal nerve and much spontaneous burning pain etc The application of a mild stimulus to this area namely the drawing of the finger nail along the skin still caused the appearance of a violent triple response which persisted and led to the development of severe inflammatory changes along the line of stimulation Three months after the onset the patient began to bathe the painful analgesic area with warm water containing dettol which had no effect on the normal skin This resulted in a violent inflammatory disturbance in the analgesic skin and caused a recurrence of œdema of the eyelid These observations seem to show that in herpes zoster all tissues superficial deep or visceral supplied by the affected posterior nerve roots may be involved that the *inflammatory tendency after herpes zoster occurs in tissues deprived of their normal sensory nerve supply and that stimuli which would not normally cause inflammation do so in such temporarily and locally denervated areas*

(2) In cases in which alcohol block or section of a sensory nerve such as the trigeminal or sciatic has been carried out there is of course at first a noticeably increased sensitivity and an exaggerated triple response of the tissues in the analgesic area to noxious stimuli and a ready tendency for these tissues to become inflamed This has been ascribed to a disregard of stimuli which would cause pain when applied to the normal tissues especially the eye Similarly in cases with damage to the peripheral nerves supplying the limbs inflammatory lesions are especially liable to occur and are exaggerated as compared with the normal in the analgesic areas so called trophic lesions This is seen particularly with lesions of the median and

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The unmyelinated posterior nerve root fibres supply not only the skin but also the deep tissues and inflammatory changes may likewise involve the deep tissues supplied by the affected nerve roots (Wyburn-Mason 1957a) Similar posterior nerve root unmyelinated afferent nerve fibres pass to the viscera The writer (Wyburn-Mason 1957a) has shown that in cases of herpes zoster inflammatory lesions are found in the viscera supplied by the affected afferent nerve roots

We have seen above that the triple response is exaggerated in the rash free skin of the affected dermatome during an attack of herpes If a line is drawn with a finger nail on the skin immediately adjacent to the lesions of herpes zoster there is a gross exaggeration of the triple response (dermographism) and the site of pressure becomes pigmented for more than a month (Souques 1921 Thomas 1925) Furthermore I have observed that such stimuli may cause the appearance of zosteric vesicles in the stimulated area which was previously free of such lesions Thus in a woman of seventy one years a subject of herpes zoster confined to the area of supply of the supra orbital nerve on one side the application to the frontal region on this side of warm water easily tolerated over the normal skin resulted within a few hours in a violent inflammatory reaction and the whole of the skin supplied by the ophthalmic division of the trigeminal nerve including the eyelids became acutely inflamed Eight weeks later after healing had occurred there remained almost complete analgesia in the ophthalmic division of the trigeminal nerve and much spontaneous burning pain etc The application of a mild stimulus to this area namely the drawing of the finger nail along the skin still caused the appearance of a violent triple response which persisted and led to the development of severe inflammatory changes along the line of stimulation Three months after the onset the patient began to bathe the painful analgesic area with warm water containing dettol which had no effect on the normal skin This resulted in a violent inflammatory disturbance in the analgesic skin and caused a recurrence of oedema of the eyelid These observations seem to show that in herpes zoster all tissues superficial deep or visceral supplied by the affected posterior nerve roots may be involved that the *inflammatory tendency after herpes zoster occurs in tissues deprived of their normal sensory nerve supply and that stimuli which would not normally cause inflammation do so in such temporarily and locally denervated areas*

(2) In cases in which alcohol block or section of a sensory nerve such as the trigeminal or sciatic has been carried out there is of course at first a noticeably increased sensitivity and an exaggerated triple response of the tissues in the analgesic area to noxious stimuli and a ready tendency for these tissues to become inflamed This has been ascribed to a disregard of stimuli which would cause pain when applied to the normal tissues especially the eye Similarly in cases with damage to the peripheral nerves supplying the limbs inflammatory lesions are especially liable to occur and are exaggerated as compared with the normal in the analgesic areas so called trophic lesions This is seen particularly with lesions of the median and

brain probably the hypothalamus Cases of alternating hemiatrophy certainly point to a central origin

Alopecia Areata and Nervous Lesions

The role of nervous disturbance in producing this condition is generally recognized In some cases the patches of baldness appear in the area of supply of a definite nerve Alopecia areata may also occur in cases of tabes Leriche (1936) reported a case in which a spinal lesion at D7 level gave rise to persistent pain in this segment and two bald painful areas of alopecia areata appeared in the occipital region and the neck below it The pain was relieved by local anæsthetization and this was followed by regrowth of the hair

Herpes Zoster, a Phenomenon resulting from Lesions of Nervous Pathways

The skin lesions of herpes zoster lie in the area of sensory supply of the posterior and trigeminal sensory nerve roots where the causative virus appears to prosper The author (Wyburn Mason 1957a) has found that not only the skin but all somatic tissues and the viscera supplied by the same sensory nerve roots exhibit lesions Lewis (1927) showed that herpetic and herpetiform eruptions occur as sequels to lesions of the sensory nerve tracts not only do they follow irritative lesions of the (root) ganglion but they are produced also by lesions of those tracts distal to the ganglia themselves The fibres concerned appear to be unmyelinated C fibres

Causalgia

This painful condition tends to follow minor lesions of the median or sciatic nerves and develops at various intervals after the injury The tissues supplied by the affected *sensory* nerves show many inflammatory changes and growth disturbances

"Central" Pain

Central pain may result from lesions in the neighbourhood of the thalamus and hypothalamus and of the central grey matter of the midbrain pons *medulla or* cord (Riddoch 1938a) It affects the tissues of the opposite half of the body which are the seat of a severe dull aching burning pain occurring spontaneously but also precipitated by stimuli of all kinds exteroceptive proprioceptive and visceral Often there is apparent analgesia of the affected part

Thus all the above conditions would appear to result from lesions of nervous pathways in the peripheral nerves or around the central grey matter of the spinal cord bulb or midbrain or of the hypothalamus

Evidence for the Inhibitory Nervous Control of the Phenomena of Inflammation

There is clinical evidence which suggests that normally nervous impulses inhibit the phenomena of inflammation

(1) In cases of herpes zoster typical inflammatory changes are found in the skin and other affected tissues As already shown *the neighbouring epidermis shows* increased numbers of vitally staining Langerhans and *clear cells as compared with*

portions of the cheek which disappeared The teeth became soft easily worn away crumbled and broke off Severance of the trigeminal nerve intensified these changes in the denervated regions of the head All the lesions described are of an œdematous and inflammatory type

At autopsy inflammation and hæmorrhages were found in the lungs which had the appearance of red hepatization Blood and mucus were present in the lumen of the whole gastro intestinal tract in the walls of which were extensive hæmorrhages and inflammatory changes Large areas of mucosa were destroyed or desquamated In other animals there appeared pyorrhœa conjunctivitis keratitis perforating ulcers of the cornea and inflammatory conditions of varying severity of both eyes which might be completely destroyed or later heal A purulent rhinitis sinusitis or otitis media sometimes developed

Several observations are pertinent with regard to the effect of the nervous system on macrophage activity Firstly it has been found impossible to produce the changes of herpes zoster experimentally in animals by *stimulating* the sensory root of the trigeminal or the posterior nerve roots Secondly a lesion of a sensory nerve root predisposes to the localization of zoster virus in the denervated tissues Thirdly the phenomena of herpes zoster may appear in the affected dermatome after a locally applied stimulus Fourthly the tendency to become inflamed in denervated tissues is exhibited in response to a stimulus which when applied to normal skin gives rise to no pain or other indications which might suggest that it is injuring the skin This disposes of the argument that such trophic sores are due to unfelt injurious agents acting on the affected tissues

On the other hand if the trigeminal sensory root is destroyed or sectioned a sensory peripheral nerve to a limb especially the median and sciatic nerves is sectioned or if the spinal cord is transected or compressed or in some patients with a lesion of one half of the bulb or hypothalamus or animals with lesions of the hypothalamus then the tissues to which the sensory nerve supply is interrupted *show an excessive triple response to stimuli and an excessive tendency to become* inflamed the response also being excessive in degree and duration We have seen that the phagocytic activity of macrophages is increased in areas of inflammation and suggested that the phenomena of inflammation result from disturbance of the macrophages of the tissues with a resultant increase in their phagocytic activity It is therefore further suggested that *the activity in some or all of the unmyelinated C fibres of any tissue presumably centrifugal in direction and descending from the hypo thalamus causes inhibition of phagocytic activity of the macrophages and restores them to the non phagocytic state*

The Nervous Control of Cell Metabolism by Way of the Reticulo endothelial System

If the deductions made above are correct it should also follow that excess of inhibitory nerve impulses to the macrophages would lead to wasting of tissues whereas if the inhibitory impulses are diminished the tissues should exhibit increased growth However when sensory nervous pathways are cut there is usually also a motor denervation of tissues which tends to cause disuse atrophy Moreover

sciatic nerves With lesions of the median nerve accompanied by causalgia the whole hand becomes swollen hot and red and there is a marked over reaction to stimuli both in the degree of pain and the production of an exaggerated triple response merging into inflammation Herpetic lesions may appear Gowers (1899) mentions that in cases of *peripheral nerve lesions with causalgia dermographism is* extremely readily obtained in the painful area and even the mildest stimuli such as warm water, applied to the affected skin may result in acute inflammation while mustard plasters cause an exaggerated response as compared with normal In a case of sciatica with hyperalgesia and sensory disturbance in the leg described by Kaufmann and Winkel (1922) the application of iodine over the affected limb caused the appearance of a dermatitis which was solely confined to the area of sensory disturbance

(3) Transverse lesions of the spinal cord may also modify the reactions of the tissues to stimuli We have seen that the triple response is grossly exaggerated in the tissues below the level of the lesion where there is of course a great tendency *for the appearance of violent inflammatory reactions with bleb formation* at sites of pressure or even in regions of mild stimulation such as contact with the bed clothes lying over the subject As with painful lesions of peripheral nerves Gowers recalls that in the tissues below the level of a transverse lesion of the cord even the mildest stimulus to the skin such as warm water may cause acute inflammation and mild pressure may lead to inflammatory changes and afterwards to gangrene In a case of traumatic acute hæmatomyelia in a man of seventy years which I observed widespread areas of acute inflammation with bleb formation developed on the abdomen and thighs and in various other regions including sites of pressure within five hours of receipt of the injury and without exposure to hot water bottles

(4) In cases of central pain the skin of the painful parts is often anæsthetic It is flushed and the tissues may be œdematous (Riddoch) In paroxysms of pain flushing increases We have seen that in the affected tissues the triple response is much more readily produced and greatly prolonged Stimuli may easily cause ulceration which fails to heal The joints may be swollen and tend to develop effusions and to become ankylosed Negro (1934) describes patients and draws attention to many recorded in the literature who developed pain and acute unilateral arthritic changes following a lesion of one side of the hypothalamus and midbrain

(5) In experiments on lower animals (dogs) Speransky (1935) and his associates introduced a glass sphere behind the tuber cinereum and between the cerebral peduncles or they encircled the infundibulum with a glass ring This produced disturbances of function in the cells of these structures Some animals died within ten to twenty hours others in a few days others survived *for some weeks and still* others for a number of months The hair on the head and face fell off The gums became swollen and loosened and showed a dark border of acute hæmorrhages around the molars and both jaws These changes spread and the gums separated from the necks of the teeth revealing the alveolar edge of bone In some cases the whole gum separated leaving the bone exposed Erosions and ulcers of the lips tongue pharynx palate and the floor of the mouth appeared They were either superficial or extended deeply destroying the mucosal muscular and cutaneous

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secretion on this side decreases The eye may remarkably diminish in size The external ear becomes smaller Partial cataract atrophy of the sclera and cornea and Argyll Robertson pupil with its accompanying loss of iris pigment may occur Diplopia due to weakness of oculomotor muscles may be a feature The superficial temporal artery may pulsate less than on the normal side Signs of involvement of the brain on the side of the hemiatrophy may be observed Epilepsy may involve the opposite part of the body only and this may also show signs of a pyramidal tract lesion Calcification may occur on the side of the atrophy and dilatation of the ventricle and atrophy of the hemisphere Oppenheim (1911) pointed out that the skin of the affected region does not flush in company with the rest of the body

In this condition there again occurs avascularity loss of pigmentation of skin and iris and loss of hair and later an atrophy of all the skin mucosal and deeper structures The skin fails to flush in response to a stimulus or when the rest of the body does so

(5) *Facial Hemihypertrophy* All the tissues in the affected trigeminal area show hypertrophy and overgrowth The epidermis is hypertrophic more pigmented more vascular greasy and sweaty The subcutaneous fat and muscles including those of the tongue hypertrophy and the bones show periosteal overgrowth The skin may be œdematous and exhibit dermographism (Cassirer and Hirschfeld)

(6) *General Hemiatrophy and Hypertrophy* In general hemiatrophy all the affected tissues become smaller atrophic and relatively bloodless and the skin loses its pigmentation as in facial hemiatrophy whereas in hypertrophy all the tissues tend to be hypertrophic the skin thicker and more greasy pigmented and vascular and there are increased fatty deposits

(7) *The Effects of Compression of the Cord on the Tissues below the Level of the Lesion* The effects of compression of the cord on the growth of tissues below the lesion is complicated by the occurrence of disuse atrophy if paralysis exists The tissues below the level of the lesion of course exhibit an exaggerated triple response and tendency to become inflamed In some cases in spite of disuse hypertrophic lesions are seen especially when transection is incomplete Thus such a lesion may cause the skin below the level of the lesion to become thickened and its hair to grow more abundantly Hyperkeratosis and dark pigmentation with a sharp upper border at the level of the lesion has been recorded In other cases the bones below the lesion may become decalcified or hypercalcified Sudeck s bone atrophy may occur below the level of the lesion (Allen Barker and Hines 1946) with subperiosteal new bone formation The case *described by Crocker (1903) is of great interest The* patient a man of 36 years suffered from Von Recklinghausen s disease and as a result of a spinal injury in the lower dorsal region sustained a paraplegia which lasted for eight months Two years after the accident the buttocks began to increase in size and continued to grow over the next ten years The legs also began to enlarge and enormous pendulous folds of skin and subcutaneous tissue overlapping like flounces hung from the level of the twelfth rib half way down the thighs and down below the knees growing from the body supplied by the lower part of the damaged cord The skin below *the cord lesion was more pigmented than the* rest of the body Sensation was apparently normal below the cord lesion

when all inhibitory impulses to macrophages are cut off the denervated tissues tend to develop acute inflammatory changes which may mask any tendency to over growth If the inhibitory impulses are not completely cut off but only diminished though the increased tendency to inflammatory changes may remain in a lesser degree the affected tissues tend to show overgrowth The study of a number of conditions already shown to be dependent on lesions of the peripheral nerves of the central nervous system points to such conclusions

(1) *Growth Changes following Causalgia* After the severe and prolonged pain of causalgia begins to die down various disturbances of growth may be visible in the affected limb (see Wilson 1940) The epidermis may become greatly thickened so that the hand looks like a washerwoman s The hair and nails grow more rapidly and the latter may be thickened New bone tends to be laid down under the periosteum Radiographs show the latter to be unevenly raised and a thin layer of newly formed bone with a serrated outline is spread over the shafts of long bones Later disuse atrophic changes occur in the tissues

(2) *Alopecia Areata* In this condition the skin atrophies and the hair falls out All the layers of the skin are thin bloodless pale and deficient in pigment Typically the affected skin fails to sweat in response to pilocarpine and as shown already when irritants are applied does not exhibit a triple response or inflammatory changes

(3) *Scleroderma* It has been shown that the affected tissues show decreased numbers of macrophages exhibiting active phagocytosis as compared with the normal though such macrophages can be demonstrated by stimulation by foreign proteins In this disease the skin gradually atrophies and becomes pale dry bluish and parchment like The sweat and sebaceous secretions disappear The hair in the affected regions turns grey and falls out The nails become brittle and ridged and their growth is slowed Fat is completely lost in the affected areas The skin becomes attached to the deeper tissues and fixation produces contractures and deformities Raynaud s phenomena may occur We have seen that the atrophic changes are not however confined to the skin but extend to the muscles bones joints intervertebral cartilages corpora mammæ mucosa of the gastro intestinal tract and bronchi lungs myocardium thyroid ovary kidney eye and bone marrow in fact to any tissue It was also shown that in the affected skin the triple response is almost unobtainable A wound in such areas leads to no reactive hyperæmia bleeds little or not at all and fails to heal normally Indolent ulcers may appear at the ends of the fingers or gangrene of the gut may develop

(4) *Facial Hemiatrophy* As in scleroderma the condition consists of a progressive atrophy of the tissues including the skin subcutaneous tissue muscle and bone (see Wyburn Mason 1950) The skin becomes thin pale parchment like and loses its pigment The hair falls out or greys The sweat and sebaceous secretions cease The fat in the affected region is lost Both the muscle and fat of the cheek disappear The nasal cartilages and bones may become smaller The jaws decrease in size on the affected side until they are too small to hold the teeth The bones are osteo porotic Half of the tongue decreases in size The mucosa of the cheek the mucous glands salivary glands tonsil and papillæ of the tongue atrophy The salivary

status quo by two mechanisms a local neural and a general hormonal by way of the adrenal cortical secretion

The onset of fatigue in cells is probably due to accumulation of metabolites We have suggested that this is prevented by the activity of macrophages The discharge of unmyelinated fibres leading to inhibition of phagocytosis by macrophages should therefore affect the fatigue of other body cells That this is so is perhaps suggested by the fact that ingested cocaine which abolishes or slows discharge in unmyelinated nerve fibres delays the onset of fatigue

During wasting diseases and starvation the various tissues waste to different degrees Presumably this differential wasting is dependent on variations in the number of inhibitory nerve impulses passing to the macrophages in the various tissues

The Control of the Differentiation and Number of Circulating Red and White Blood Cells by the Nervous System

The cardio vascular and respiratory systems concerned with the transport of oxygen and other substances to the tissues are controlled in the hypothalamus and bulb It might be expected therefore that the formation of red blood corpuscles would be similarly controlled and if this is so the formation of white blood corpuscles also

The number of red and white cells per cubic millimetre of blood is maintained fairly constant during health The blood count depends on a number of factors The state of contraction of the spleen which is controlled by nervous impulses is one such The lymph nodes also have a plain muscular capsule and are supplied with autonomic fibres which evidently control the contraction of the nodes The expulsion of the blood cells from the lymphatic node is thus a vasomotor reaction involving the communication of the blood sinuses of the nodes with the general lymph circulation It has been suggested that the expulsion of the cells from the bone marrow is also a vasomotor nervous reaction (Best and Taylor 1950)

The lymphocytes are produced in lymphoid tissue present in lymph nodes thymus spleen and in the wall of the gastro intestinal tract whereas red blood corpuscles and the myeloid series of white cells are formed in the bone marrow Unmyelinated nerve fibres accompanied by macrophages enter the hilum of the lymphoid tissue and then pass round the margins of the germinal centres in relation to the syncytium of littoral macrophage cells Only those at the edge in relation to the nerve fibres normally stain vitally In the reticulin network there are also found primitive hæmopoietic cells which develop into lymphocytes The reticulo endothelial cells adsorb substances from the blood and pass them to the developing blood cells Alterations in nervous discharge will affect the phagocytic activity of the local macrophage syncytium and thus the rate of growth and differentiation of the parent cells of the lymphoid series lying on the reticular fibres may be changed Similar conditions exist in the bone marrow Red and white cells evolve by a process of differentiation from precursor cells lying in the reticulin on which lie also the littoral macrophage syncytium The function of this syncytium must depend in part on nerve fibre activity to the reticulo endothelial system in the same

All the above conditions have been *shown to result from lesions of the central* nervous system or of the posterior spinal or trigeminal *sensory* nerves. The hypertrophic and atrophic changes are confined to the *sensory* dermatomes or sensory supply of a peripheral nerve and not to vascular areas. Moreover, vasodilatation alone as produced by sympathectomy does not lead to hypertrophy of tissues so that these growth changes are to be related to disturbance not in vascularity or *autonomic innervation but in* posterior or trigeminal *sensory* nerve root innervation of the affected tissues, like the variations in the triple response and repair of injuries in these areas.

In all the diseases considered the phenomena observed in the affected tissues may fall into two groups: (1) lesions like hemihypertrophy and those below the level of a transverse lesion of the cord exhibiting vasodilatation with over reaction to noxious stimuli as manifested by exaggeration of the triple response and inflammatory reaction to stimuli attributable to increased excitability of macrophages. As shown already this state appears to be related to a cutting off of nervous impulses passing to the macrophages by which their phagocytic activity is normally inhibited. It is accompanied by overgrowth and hypertrophy of tissues and over production of fat and pigment. (2) Lesions like scleroderma, hemiatrophy and alopecia areata showing vasoconstriction with little or no triple response or inflammatory reaction to noxious agents and little attempt at reparative reaction, poor healing of injuries, atrophy of the tissues, disappearance of fat and failure to form pigment.

These observations suggest that a centrifugal discharge of impulses emanating from the hypothalamus and passing down the cord and to the peripheries via the posterior nerve roots causes an inhibition of phagocytic activity in the macrophages of the various organs. When in excess these impulses result in inhibition of vital staining, vaso constriction, failure or diminution of inflammatory response and proliferation in response to injury. In addition these changes are accompanied by atrophy of various tissues and failure to form pigment. Diminution in the number of these inhibitory impulses leads to the opposite changes with vasodilatation and a *tendency to inflammation and is also associated with* hypertrophy of the tissues and excessive fat and pigment formation. Presumably the growth changes like those in response to noxious stimuli are dependent also on alterations *in the phagocytic activity* of macrophages. This activity of nerve fibres is in fact trophic in nature. It seems therefore that through the medium of the reticulo endothelial cells under nervous control the metabolism of various tissues is correlated with others *and the general metabolism is controlled.* The macrophages bring the endocrine secretions to bear on their target cells. Control of the activity of the reticulo endothelial cells by *autonomic* nerve fibres seems to have been suggested by Thomas (1949) and by Morin and Poggio (1949). Moreover Zander and Weddell (1951) observe that the plexiform arrangement of nerve fibres in relation to the corneal corpuscles suggested that they perform other functions than subserving sensation, for example that of nocifensor nerves. The idea that certain posterior nerve root fibres discharge centrifugally is of course contrary to Bell's law and revives a similar suggestion made many years ago. Once the functional state of macrophages has been altered locally by the application of a stimulus it tends to be restored to the

that the rate of cell division and differentiation of all tissues is dependent on nervous impulses The finer adjustments of the number of circulating cells depends on the autonomic system Depression of inhibitory nervous discharge in relation to the reticulo endothelial cells of the hæmopoietic organs would result in hyperplasia in these tissues and a rise in the number of cells and their precursors in the blood while increased nervous discharge has the opposite effect leading to fibrosis in the organs with a fall in the number of circulating blood cells of appropriate kinds Established polycythæmia with its associated leucocytosis may well be due to a permanent acquired or congenital lesion of the hypothalamus This has been suggested previously by a number of workers Conversely some cases of anæmia may be due to disturbance of hypothalamic function

The Control of Neuronal Function within the Nervous System by the Microglia and its Relation to Mental Disease

As in other tissues the metabolism of the neurones within the nervous system appears to be controlled by the macrophage microglial cells Like macrophages elsewhere these are under the inhibitory influence of unmyelinated nerve fibres the central control of which presumably lies in the hypothalamus The activity of the microglial cells is altered by many agents such as drugs toxins trauma etc Clinically all of these may produce every variety of mental disturbance such as mania depression confusion schizoid states or stupor identical in character with those occurring without apparent cause Lesions of the hypothalamus may also give rise to symptoms identical with those caused by noxa Moreover cortisone which appears to act primarily on macrophages may produce euphoria depression or more serious disturbances such as confusion character changes or psychoses It seems possible that in all such organic conditions the essential cause lies in the disturbed activity of the macrophages (microglia) controlling neuronal function within the brain as a result of the action of the noxious agent or of disturbance of the controlling inhibitory nerve fibres In mental disturbance arising without obvious cause intercurrent illness head trauma etc may temporarily restore the mental state to normal The basis of the symptoms may lie in an abnormality in the inhibitory nervous control of the microglia by unmyelinated nerve fibres emanating from the hypothalamus

way as the differentiation of the cells of other tissues The existence of such nerves controlling the hæmopoietic tissue and the differentiation of blood cell precursors into the mature cells of the circulating blood is shown clinically by the following —

(1) In cases of facial or general hemihypertrophy which are due to diminished inhibitory nervous discharge to the reticulo endothelial cells in the affected areas, the tonsil and lymph nodes like other tissues are larger on this side

(2) In the affected areas of scleroderma or facial hemiatrophy which are apparently due to excessive inhibitory nervous discharge to the reticulo endothelial cells in the affected regions, the lymph tissues are atrophic and fibrous and there may be a microcytic anæmia due to atrophic changes in the bone marrow

(3) *In cases of herpes zoster the lymph nodes and bone marrow (Schleicher 1949)* may show inflammatory and proliferative changes even before the appearance of the eruption and secondary infection (Wyburn Mason 1957a) suggesting loss of inhibitory nervous control of function of the reticulo endothelial cells in these as in other affected tissues

(4) Lesions of the walls of the third ventricle such as hypothalamic or third ventricle tumours or encephalitis lethargica may also result in alterations in the *blood cell count (Riddoch 1938b) Such patients may show polycythæmia leucocytosis* eosinophilia or lymphocytosis Lesions of the hypothalamus giving rise to polycythæmia up to 9 000 000 cells per cu mm and white cell counts as high as 50 000 per cu mm were recorded by Guillain Lechelle and Garçin (1932) Moehlig and Bates (1933) Laruelle (1934) Lhermitte (1934) and Haynal Graf and Matsch (1949) (For review see Carpenter Schwartz and Walker 1943) These may disappear after removal of a basal tumour Polycythæmia may occur with subtentorial *tumours or subdural hæmatomata and disappear after their removal (Carpenter* Schwartz and Walker 1943 Drew and Grant 1945 Lhermitte 1934) It may result from a lesion of the basal ganglia following carbon monoxide poisoning (Dittmar 1939) acquired hydrocephalus (Primrose 1952) encephalitis lethargica (Schulhof and Matthies 1927 Silus 1933 Moehlig and Bates 1933) from Huntington s chorea (Schulhof and Matthies 1927) and concussion (Hecht and Weil 1929) and may accompany narcolepsy (Gunther 1930) which arises from a *hypothalamic disturbance Stab wounds in the region of the corpus striatum and* hypothalamus may also cause a neutrophil leucocytosis (Rosenow 1928) while *diathermic stimulation in the region of the third ventricle may lead to a polymorph* leucopenia (Wossidlo 1935)

Cerebral tumours especially those involving the basal ganglia tend to cause lymphocytosis or eosinophilia (Morawiecka 1927 Geerling 1930) Traumatic intracranial lesions may likewise cause an immediate increase in the white cell *count (Wright and Livingstone 1953) Clinical experimental evidence for the regulation* of erythroplasia by the central nervous system has also recently been brought forward by Haynal Graf Matsch Cseley and Edelys (1950) The influence of the nervous system on hæmopoiesis and regulation of the leucocytic count was likewise shown by Wachholder and Neuberg (1950) and Thalhammer and Jancek (1951)

It must be concluded that the rate of formation of blood cells in the hæmopoietic system is under nervous control This is merely an example of the general rule

The Influence of Nerves in Regeneration of Parts of Vertebrates and Invertebrates

In worms and other invertebrates the cut ends of nerves have a trophic influence on the regeneration of excised parts (Berrill 1931 Schotte 1926 Locatelli 1929 Abeloos 1932 and others) The nervous system has been found to play an important part in the regeneration of the earth worm for the nerve cord must be present at the cut surface if regeneration is to take place from that surface If the anterior end of a worm is cut off and in addition the nerve cord is extirpated for a short distance behind the cut surface an anterior end may be regenerated from the place where the nerve cord ends but never from the original cut surface (Morgan 1902 Huxley and de Beer 1934)

In vertebrates such as fish and many amphibia if the limbs or the tail are severed the part regenerates The subject has been well reviewed by Singer (1952) Todd in 1823 reported that interruption of the sciatic nerve profoundly affects regeneration of the hind limb of the salamander if the division of the nerve be made after healing of the stump reproduction is either retarded or entirely prevented And if the nerve be divided after reproduction has commenced or considerably advanced the new growth either remains stationary or it wastes becomes shrivelled and shapeless or entirely disappears Singer has shown that the regeneration of removed parts depends on the integrity of the nerve supply of the stump both in the larva and adult and that only the sensory components that is the posterior nerve root fibres were the important ones There is no accumulation of mesenchymatous cells at the amputation surface of a larval or adult amphibian to form a blastema when denervation is performed at the same time as amputation (Schotte and Butler 1941) The nerve is essential for the initiation of regeneration and acts as a regulating agent of the complex cellular interactions which are responsible for regeneration

In man when a large peripheral nerve such as the sciatic is severed the peripheral cut ends of the nerve degenerate and the macrophages around the fine nerve branches disappear As shown already the affected tissues show no triple or inflammatory response to noxa The denervated tissues exhibit trophic changes and may show perforating trophic ulcers which may involve bone and fail to exhibit any attempt at regeneration

In cases of tabes and leprosy in man the posterior spinal root ganglion cells and their axons degenerate In advanced cases of tabes all sensation may be lost in various parts of the body In late stages the skin atrophies There is generally a loss of subcutaneous fat which may be extreme (marantic tabes) The buccal mucosa may atrophy and indolent perforating ulcers appear or teeth become carious and fall out The eye may exhibit optic atrophy atrophy of any part of it or general atrophy of the globe The Argyll Robertson pupil with its loss of iris pigment is usual Osteoporosis affects the bones leading in time to destruction of their articular ends and even beyond Bits of cartilage may become eroded and pieces of bone rarefy disintegrate and become detached The bony architecture is deformed and weakened Dislocations subluxations and painless fractures which fail to unite are common There is no redness inflammatory reaction or pyrexia The gut

CHAPTER VII

THE INFLUENCE OF MACROPHAGES ON REGENERATION OF PARTS IN VERTEBRATES AND EMBRYONIC DEVELOPMENT

The phenomena of inflammation are followed by and merge into those of regeneration The regeneration process bears many resemblances to embryonic development A striking similarity is the accumulation in the region of injury of cells which have the disposition and appearance of the mesenchymal cells of the embryo concentrations of which give rise to various organs Moreover the rapid growth of this cellular mass and its later structural and functional differentiation resemble closely embryonic formations in general appearance arrangement and developmental sequence The regenerate arises directly from and develops in intimate relation with adult structures and there is no barrier between the new formation and the old tissues The histology of the regenerate emphasizes the continuity and inter relation between adult and regenerating tissue the former passing gradually into the latter with no absolute line of demarcation between the two Moreover regeneration occurs in typical fashion in the immature animal or in the developing embryo

The regenerating tissue throughout its development is nourished by the adult blood stream and there is no apparent obstacle to free exchange between the two unless it be endothelial in nature Thus the new growth is perfused continuously with substances of adult origin and importance Macrophages appear very early in the area in all these conditions In the case of wounds or injury there is an increase in the vital staining of the area and mobilization of resting macrophages and pericytes An interstitial matrix is then laid down by the fibroblasts and in this reticulin fibres are first to appear The next change in regeneration of connective tissue is a budding of the capillary endothelial cells at the margin of the injured area and an outgrowth of capillary sprouts accompanied by pericytes into the matrix and then the development of collagen fibres The cells of regeneration have their origin either directly from adult cells by a series of changes in which the cell divests itself of differentiated structures or from primitive cells which lie among the adult tissues and are called forth by the injury (review by Weiss 1939 Schotte 1940) The cells gradually become transformed into fibroblasts lipoblasts angioblasts chondroblasts osteoblasts etc Regenerating nerve fibres arising from the interrupted ends of adult neurones invade the regenerate and are present within it in great numbers at all stages of development

We have seen that cortisone appears to depress the activity of macrophages It also inhibits the formation of fibres new vessels and granulation tissue and suppresses the reaction to repair (Dunlop 1955) Such observations suggest that the activity of macrophages is essential for regeneration of tissues

substances to places where they are required removing waste products which arise in physiological wear and tear of tissues and reconstituting the growth of tissue where needed In view of the similarity of embryonic development to regeneration it seems probable that local macrophages play the same role in the two conditions They must help to concentrate circulating physiological substances in the area and make them available to the multipotential cells of the regenerating tissues They also remove débris and metabolites If a normal tissue is injured there follows the immediate reaction known as inflammation and this merges into that of regeneration Where one process ends and the other begins is impossible to say and often regeneration is considered as part of the phenomena of inflammation It seems that both are dependent on the same stimulation of macrophages of the damaged tissues Thus can be explained the stimulation of subperiosteal bone growth that occurs in the region of inflammation affecting bone

After removal of a part of the body either a part or the whole of a limb the breast or a smaller structure or even after a lesser injury it is usual to find that there is an overgrowth of the distal cut end of the nerves running to the part They are swollen and simulate a tumour Such an amputation neuroma however always develops to some extent after removal of only a very small part For example such so called amputation neuromata may be found in the base of chronic peptic ulcers chronic tuberculous ulcers or in the bile or cystic ducts in long standing cases of gall stones or ulcerative cholangitis (see Feyrter 1948)

It would seem that in all cases in which there is damage to a part of the body or a part of the body however small is removed that there occurs a division and a proliferation of the macrophages which pass into the regenerating tissues The growing tips of the nerve fibres later reach the macrophages The macrophages serve to concentrate physiological substances in the redifferentiating areas

The Neural Crest and Embryonic Differentiation in the Vertebrate

As development of the blastoderm of reptiles mammals and birds proceeds the primitive streak appears in the dorsal midline It represents the blastopore of lower vertebrate ova From the sides of the streak the lateral mesenchyme is largely derived and grows forward and laterally This consists of stellate multipotential cells lying in a mucinous matrix From the anterior end of the primitive streak cells grow forward and differentiate into the neural folds the notochord and mesoderm The neural folds run parallel to the long axis of the embryo on either side of the mid dorsal line and rise up and fuse in the mid line Before they fuse the neural crest differentiates and forms cells at the summit of the neural fold and its cells migrate into all parts of the body

As soon as the primitive multipotential mesenchyme cells appear and begin to differentiate there are seen among them vitally staining macrophages (Maximov 1909) and fine argyrophil reticulin and later collagen fibres Macrophages appear very early in embryonic life New formation occurs from these around blood vessels nerve trunks and in the tissues generally In the middle of embryonic intra uterine life the loose connective tissue is so rich in macrophages that it resembles the inflamed connective tissue of adults (Maximov 1909) The primitive multipotential

becomes sluggish insensitive and atrophic the bladder insensitive and thinned and may become gangrenous and perforate Indolent painless perforating ulcers frequently develop usually on the feet In the region of the ulcers the bone may become rarefied and necrotic and the pieces extrude through the ulcer We have shown above that both nerve fibres and macrophages have been shown to be absent *in these areas and that in the region of such necrotic ulcers the triple response* could not be elicited

In cases of leprosy there are also found similar atrophic changes in the peripheral nerve axons The skin atrophies The hair becomes brittle and is shed At the limb extremities a panatrophy involves nails skin hair subcutaneous tissues muscle and bone Terminal phalanges become thickened and shortened rarefy and eventually are adsorbed Perforating ulcers open sores through which fragments of necrotic bone are extruded and arthropathies develop They resemble those of tabes There is no inflammatory response or attempt at regeneration after damage by noxa which instead causes necrosis Similar changes are found in the buccal and nasal mucosa and the bones and tissues of the face are involved The similarity of the changes to those occurring in tabes is obvious

In normal conditions the macrophages are congregated around the finest branches of the unmyelinated nerve fibres In all the above clinical conditions these nerves have disappeared and macrophages are no longer present in the tissues in any numbers An injury to the skin or deep tissues including bone fails to heal is painless and results in little or no inflammatory response and no triple response to noxious stimuli Instead injury leads to necrosis and this may spread There is no attempt at regeneration and redifferentiation of tissues All these phenomena would seem related to the absence of macrophages in the tissues

This view agrees with that of other workers who believe that the development of granulation tissue is apparently dependent on the initial activity of the macrophages (see Franceschini 1955) Oberling and Boucin (1931) for example followed the effect of blockage of the reticulo endothelial system on the regeneration of wounds in rats and concluded that it markedly interfered with the wound healing Again after surgical removal of a portion of liver or when large numbers of liver cells *are killed by disease or intoxication regeneration occurs frequently* Before this takes place there is an outgrowth of Kupffer cells into the tissue spaces where reticulin fibres are formed (Florey 1954) This seems to be an essential phenomenon The perichondrium and periosteum have been shown to control the nutrition of the cartilage and bone cells respectively In regeneration, as in embryonic development of bone the periosteum with its macrophages is essential Carrel (1922) and his fellow workers (Carrel and Ebeling 1926) showed that macrophages exert a *trophic* action on other cells which reveals itself during tissue proliferation and regeneration He found that they promote the multiplication and differentiation of epithelial cells and tissue culture fibroblasts They may stimulate the growth of tissues or organisms Jaffe (1938) also thinks that macrophages may stimulate the growth of connective tissue and of covering epithelium Carrel (1924) observed that free macrophages serve apparently in the nutrition of the inflamed tissue by carrying

medulla the sympathetic ganglia in the retroperitoneal region on the aorta in the prevertebral ganglia of the sympathetic and in the sympathetic plexuses such as the coeliac superior and inferior mesenteric the tympanic body in the heart (Wiessel s cardiac paraganglion) kidney renal pelvis ureter liver (Maximov and Bloom) uterus testis epididymis prostate ovary (Maximov and Bloom) and paroöphoron The most prominent paraganglia in man are two aortic bodies lying anterior to the aorta in the region of the inferior mesenteric artery Chromaffin cells are always found in relation to nerve fibres and usually in relation to vessel walls

(7) *The chromatoblasts* In mammals pigment producing cells or melanoblasts normally occur in the epidermis hair and eye (iris retina and choroid) Sometimes they are also found in perivascular regions in the leptomeninges or brain on the cerebral (pial) vessels in sensory organs in the sympathetic nervous system adrenal medulla and peri coelomic tissues and in the intestinal wall and gall bladder (see Masson 1948 Dushane 1948) All pigment producing cells appear to be neural crest in origin Removal of the appropriate region of the neural crest tissue in vertebrate embryos (amphibia reptiles birds and mammals) causes failure of the pigment producing cells to appear in the skin hair mucosa or eye (iris retina and choroid) If the limb buds of an embryo are grafted heteroplastically at an early tail bud stage the limb acquires host pigmentation if at a later tail bud stage it acquires donor pigmentation In the former case the migrating cells from the neural crest have not reached the bud at the time of transplantation but in the latter have already entered the graft

(8) The *satellite cells* of the ganglion cell bodies of the posterior root ganglion and the similar capsule cells of the autonomic ganglia and their homologues and the *sheath cells of Schwann* (neurilemma cells) with which they are continuous by their processes Schwann and satellite cells both yield macrophages in tissue culture (Weiss and Hsi Wang 1945)

(9) Some at least of the *endo and peri neural cells* which are macrophages and fibroblasts are also derived developmentally from the neural crest cells (Horstadius)

(10) *Many of the cells of the meninges* The pia arachnoid (leptomeninges) is continuous with the endo perineurium and like it contains fibroblasts and macrophages the latter especially common along the neuro vascular channels If the neural crest is removed early in development the pia arachnoid shows atypical and defective development A varying number of melanoblasts can often be found in the pia arachnoid and around the cerebral vessels and these are of course derived from the neural crest

(11) *Macrophages* Many workers have concluded that the interstitial cells of Cajal are neural in type and origin and claim they are continuous syncytially with the Schwann cells which are undoubtedly neural crest in origin Again much evidence in favour of a neural origin of the Hellen Zellen cells and cells of Langerhans of the epidermis has been adduced It has been demonstrated already that the interstitial cells of Cajal the Hellen Zellen of Feyrter the basket cells and the cells of Langerhans in the epidermis are really macrophage in nature Furthermore the satellite and Schwann cells the endo perineural cells and many of the cells of the meninges are all macrophage in type and are all of neural crest origin There is

mesenchyme cells gradually become transformed into fibroblasts, lipoblasts angioblasts chondroblasts osteoblasts and blood cell precursors

The cells of the neural crest are indistinguishable from other embryonic cells during their period of migration Yet they have reached all regions of the chick embryo by the fourth day of incubation and all regions of the mouse by the twelfth day of gestation The cells migrate (1) laterally just beneath the epidermis (2) dorsally to lie above the cord (3) ventrally in fairly coherent sheets on both sides of the cord between it and the myotomes to reach the region of the ventral wall of the neural tube and notochord They pass even more ventrally in relation to the aorta and cardinal veins and ducts of Cuvier to form the cells of the sympathetic *ganglia primitive sheaths of nerves and chromaffin bodies* with further outposts moving into the viscera such as the heart lungs liver kidney adrenal gonads etc The neural crest derivatives are thus often perivascular

A good review of the development of the neural crest and its derivatives is given by Hörstadius (1950) (see also Huxley and de Beer 1934) It has been shown that the neural crest gives rise to a large number of cells in different parts of the body many of which were not previously known to be derived from it The cells directly derived from the migrating neural crest cells are —

(1) *The cranial sensory nerve ganglia and their nerve cells and fibres* (part of which may also be contributed by the placodes of lateral ectoderm of the head)

(2) *The dorsal spinal root ganglia and their nerve cells and fibres*

(3) *The sympathetic and parasympathetic ganglia* (? also from the ventral part of the cord) The ganglion cells are surrounded by satellite and mantle cells and their axons by Schwann cells

(4) *The enteric and visceral ganglia neuroblasts* including the cells of the local nerve plexuses of the lungs heart gut uterus glands etc The ganglion cells of the sympathetic are normally surrounded by satellite (capsule) and mantle cells and their axons by Schwann and endo perineural cells

(5) *The Rohon Beard cells* These are giant ganglion cells forming sensory pathways in the dorsal part of the cord in embryos and larvæ of lower vertebrates (fish and amphibia) After bilateral removal of the neural crest these cells are lacking in the corresponding regions of the cord (Hörstadius) The primitive neural crest cells evidently migrate into the central nervous system It is possible that the same phenomena occur in mammals

(6) *The chromaffin (or argentaffin) cells* Developmentally all the cells destined to become chromaffin cells originate in the neural crest though they cannot be differentiated from other cells of nervous origin in the sympathetic primordia in early embryos They are the large cells of the sympathetic ganglia They differentiate before the nerve cells of the sympathetic although they arise from the same blastema as the latter They assume the characteristic appearances of chromaffin elements relatively late during embryonic development (see Kuntz 1953) In developing fishes and other lower vertebrates a group of chromaffin cells (para ganglia) is thrown off from each ganglion of the sympathetic chain and comes into contact with the tributaries of the cardinal veins Some remain with the sympathetic ganglia In adult humans chromaffin cells are found in the adrenal

from a very early stage and during the early stages of development of the central nervous system no blood neural barrier exists Immigration always occurs in relation to the first blood vessels and to the attachment of the choroid plexuses and meninges The microglial cells invade perivascularly especially in certain areas the three fountains or nests where the pia comes into close contact with the white matter These are at the points of entrance of the choroid plexuses of the third and fourth ventricles and around the leptomeningeal vessels of the interpeduncular fossa (Ramon y Cajal 1933) The cells also enter beneath the meninges related to certain nerve tracts which are developing about the same time as the migration takes place They are the site of formation of the cerebellar peduncles at the margins of the internal capsule in the fornix and corpus callosum around the optic tracts and in the rhinal fissure They also enter at the site of attachment of the posterior nerve roots to occupy the whole ipsilateral half of the cord and also with the meningeal blood vessels (see Kappers 1929 Rydberg 1932) At the time of microglial migration the nervous system consists only of undifferentiated neuroblasts and spongioblasts from which astroglia oligodendroglia and ependymal cells arise The amount of microglia in the developing central nervous system is directly proportional to the stage of development of the neuraxis

Since many of the cells of the pia arachnoid including macrophages seem derived from the neural crest and microglial macrophages invade from the pia arachnoid then presumably microglia is also ectodermal and of neural crest origin

(13) Cells of the cranial neural crest extend ventrally into the region of the visceral arches They give rise to some of the cells of the *mesenchyme of the head region* (Hörstadius) If the neural crest of the head is removed bilaterally early in development the visceral arch cartilages chondrocranium and membrane bones of the skull teeth nasal sacs auditory vesicles and lateral line sense organs fail to form normally By following the migration of vitally staining neural crest cells these appear to become directly converted into cells of the head cartilages and visceral skeleton (Huxley and de Beer)

The Effect of Macrophages on Embryonic Differentiation

All the fundamental phenomena of morphogenesis and histogenesis are independent of those physiological actions of the nervous system which we call conducted impulses or excitations The specific character of all organs is determined long before nerves make their appearance Histological differentiation of considerable perfection has been obtained in tissue culture and in transplantation that is in conditions in which no nervous influence could reach the tissue Thus the major traits of differentiation do not require the presence of nerves Even those tissues which later come under the most direct control of the nervous system the muscles and sense organs develop first in full independence Explanted myoblasts differentiate into contractile muscle fibres (Olivo 192› 1928 Holtfreter 1929 1933) with cross striation (Goss 1933) The capacity of self differentiated heart muscle to pulsate in the absence of all innervation has been the most convincing test of the purely muscular origin of the heart beat Limbs which have been prevented from

direct experimental evidence that all macrophages are so derived Clark and Clark (1930) studied the first appearance of macrophages in the tail of very early amphibian larvæ and axolotl larvæ In axolotl larvæ they examined the tail when represented by only a small pointed bud containing a thick opaque central mass and two narrow dorsal and ventral fin folds each composed solely of two layers of epidermis This is at a stage prior to the formation of blood capillaries in the tail fin and the main vessels have not yet reached the region The cells can thus not have been blood borne Later some cells migrate from the blood vessels *In the central mass four* longitudinal divisions could be distinguished

(i) Lying dorsal to the cells which become the cord was a strand of cells at first without cell boundaries and from which cells migrate to the dorsal fin at an early age
(ii) A dorsal strand which becomes the caudal portion of the spinal cord
(iii) A central strand becoming the notochord
(iv) A ventral strand at first apparently undifferentiated with no cell boundaries and containing yolk *This resembles (i) and from it cells migrate into the ventral fin Strands (i) and (iv) give rise to pigment producing cells and* macrophages It was found that three types of macrophages arose from it (a) histoid wandering cells (b) macrophages which migrate to the fin margin and eventually become chromatophores (pigment bearing macro phages) (c) cells which form the canalicular (lateral line) apparatus

In the later stages of fœtal life the majority of the histoid wandering cells (macro phages) *pass into a resting stage (resting wandering cells) and may either persist in* this form or assume the appearance of fibroblasts Thus it seems that the first macrophages of the body originate around the cells which form the neural folds and in the region of the neural crest cells and are found in the paths of migration *of these cells in embryos With other cells such as chromatophores* which are also of neural crest origin they migrate outwards to the peripheries Thus macrophages appear to be of ectodermal rather than mesodermal origin as is usually thought a suggestion in accord with the meso ecto dermal nature of many other mesenchymal elements first shown by Platt (1893–97)

Such conclusions may explain why after bilateral extirpation of the arch neural crest the healing of wounds often proceeds very badly (Hörstadius) This is presumably due to the failure of neural crest macrophages to pass to the wound in sufficient numbers

(12) *Microglia* In the central nervous system the phagocytic and vitally staining microglial cells do not originate within the nervous system but migrate into it early in development from the leptomeninges The development of microglia is described and reviewed in an excellent article by Kershmann (1939) Van Santha and Juba (1933) showed that in rat embryos the earliest appearance of microglia is related to the first evidence of vascularization of the central nervous system In chick embryos microglial cells begin to invade the brain on the fourth day of incuba tion Kershmann identified the cells in human embryos of 5–8 mm Macchi (1949) showed that microglial cells are present in the central nervous system of chick embryos

along developing fibres forming a tubular sheath (neurilemma) around them Inside the sheath the cells (cells of Schwann) preside over the deposition of myelin If the neural crest is removed as soon as it appears motor fibres fail to migrate into the limb muscles normally Sheath cells appear to constitute an essential element in the environment for normal development and distribution of motor fibres to limb muscles The formation of myelin depends on contact of Schwann cells and nerve fibres Whether an axon becomes myelinated or not depends on the property of the axon and not on the Schwann cells It appears that neural crest cells in the shape of Schwann cells act as pilot or pathfinder cells and migrate into the developing tissues to which they guide the outgrowing nerve fibres They further serve a trophic function in relation to nerve fibres and control their differentiation outgrowth and myelin formation Axons cannot exist in the absence of these cells The homologous capsule cells control the function of the ganglion cell bodies

(2) Kershman (1939) obtained evidence which suggested that collections of microglial elements explained the direction of growth of nerve fibres in the developing central nervous system by chemotaxis of nerve endings and in fact that the microglia acts on the nervous system like the corresponding Schwann cells in the developing peripheral nerves serving to control growth and differentiation

(3) The pia arachnoid contains many macrophages and receives cells from the neural crest If the neural crest is removed early in development the pia arachnoid (leptomeninges) shows atypical and defective development This suggests that the macrophages migrating from the neural crest may be the essential cells necessary for the normal development of the pia arachnoid

(4) In the developing thyroid of vertebrates are found the so called grey cells parafollicular cells or macro thyreocytes described by various workers (Godwin 1937 and others) which also stain vitally with intra vital dyes They persist in the adult as macrophages in the interstitial tissue (see Bargmann 1939) These cells often lie tightly pressed against the developing follicles They have been shown to exercise an influence on the development of the follicles

(5) For the normal embryonic development and differentiation and continuous health of both cartilage and bone and for reformation of the latter after injury the perichondrium and the periosteum and endosteum with their contained macrophages are essential Cappell (1929) describes the presence of free macrophages in close apposition to the bony lamellæ in the developing bones of young animals They serve a trophic function in relation to developing bone

(6) Again the neural crest is necessary for the formation and thickening of the corium The migrating neural crest cells in contact with the ectoderm of the embryo cause increase in the number of cell divisions and thickening of the epidermis (Wagner 1949) The melanoblasts of the epidermis have been seen to be derived from the neural crest The only other cells of the epidermis which might be of neural crest origin would appear to be vitally staining clear cells and cells of Langerhans which seem to be macrophages and to which nerve fibres are in close relationship Thus the macrophages are perhaps essential for the normal growth and differentiation of the corium

Head neural crest cells appear to migrate to and be converted directly into

receiving nervous outgrowths nevertheless develop normal musculature (Hamburger 1928) Consequently the stimulus of functional activity, which is ordinarily mediated to the muscles by their nerves is irrelevant for muscular differentiation Sensory organs can likewise develop in the absence of innervation (Stone 1933) So can bones and joints in explanted limb buds joints form in their typical positions notwithstanding the absence of all movement (Fell and Canti 1934) After the joints have formed the bones fuse again owing to the lack of movement

It has been shown that the cells of the neural crest of the head region are essential for the differentiation and development of the nasal sacs placodes of the head the sucker and balancer organs in the head and external gills in lower vertebrates the eyes the teeth the auditory vesicles lateral line organs and taste buds (which are related developmentally and in evolution) and the visceral arch cartilages chondrocranium and mesenchyme of the head the neural crest cells being converted directly into these structures Apart from the direct conversion of neural crest cells into the cells of the various head structures from numerous observations (see Hörstadius) it has been deduced that the migrating neural crest cells exert influences on the growth of other cells and tissues If a length of the neural crest is removed as soon as it appears the tissues which normally receive the migrating neural crest cells partially or completely fail to develop Thus crest cells are essential for the formation and thickening of the corium and epidermis (Wagner, 1949) Again the cells of the trunk neural crest migrate in the mesenchyme to the outer side of the myotomes and into the dorsal and ventral fins (Raven 1931) The trunk neural crest is necessary for the formation of the fins both mesodermal and epidermal elements If only a short portion of the trunk neural crest is removed opposite the area where the dorsal fin appears a corresponding gap is said to occur in the dorsal fin during development The elongation and stretching of the notochord and musculature and the metameric arrangement of the latter are likewise dependent on the presence of the trunk neural crest cells In the absence of the neural crest the initial elongation of the tail bud stops and regression of growth sets in (Huxley and de Beer) so that the differentiation of the tail seems dependent on the neural crest cells

We have seen that macrophages are present in large numbers in all tissues from a very early stage of development In view of the deductions already made with regard to the role of macrophages in normal and degenerating tissues and the similarity of developmental and regenerative processes it would be expected that the macrophages were essential for the feeding of the developing and differentiating cells of the embryo The possibility that macrophages may influence the growth and differentiation of other cells was first demonstrated when colonies of macrophages or monocytes from the spleen were planted adjacent to colonies of fibroblasts in the plasma coagulum (Carrel 1924) The wandering cells from the spleen survived and multiplied in the plasma alone in this way differing greatly from the fibroblasts which normally do not multiply in this medium When the two groups of cells were planted together it was found that the fibroblasts also multiplied rapidly The macrophages played the role of collecting the serum constituents into food for the fibroblasts Their role in the embryo is shown in other ways

(1) In the embryo neurilemmal sheath (Schwann) cells normally group themselves

mutual inductive influences on their later development and differentiation Thus the brain and notochord mutually induce one another In the development of the teeth the dental lamina which is an ingrowth of the oral epithelium into the sub jacent mesenchyme becomes thickened in certain areas over condensations in the underlying mesenchyme The thickenings form the enamel organ the condensations in the mesenchyme the dental papillæ The cells of the latter are odontoblasts Dentine is only formed under the influence of odontoblasts in contact with oral ecto or endoderm (Holtfreter and Hamburger 1955) On the other hand enamel is only formed by the ectodermal enamel organ in contact with a tooth papilla Tyler (1955) demonstrated that the forces responsible for the specific adhesion or non adhesion of cells and tissues that is attraction during development appear analogous to antigen antibody reactions and through this are related to genic action Thus when dis sociated cells of different species are mixed in a medium coalescence is found to occur only between those of the same species It is also possible that natural auto antibodies may act directly as inductive agents *The migration of neural crest cells to their definitive position the attraction to one another of the cell elements forming the teeth and kidney the growth into a tissue of nerve axons and the attraction of macrophages to a developing part presumably depend on such processes* It follows that should genetic mutation occur during development these forces of attraction and induction between cells would be disturbed and major developmental anomalies might arise such as errors of neural crest cell migration or innervation of tissues or failure of two parts of the teeth primordia or kidney primordia to grow together

mesenchymal cells but in addition it seems probable that elsewhere *macrophages including their specialized form the Schwann cells are neural crest cells which migrate to all parts of the body and are essential for the normal growth and differentiation of the various tissues* These cells pass to and settle down in all tissues where they control the metabolism of the cells by adsorbing circulating food substances passing them to the growing cells and receiving their waste products When the neural crest is removed over an area the macrophages which would normally migrate from the removed areas fail to pass to the developing tissues *such as the pia arachnoid* skin teeth fin etc so that the differentiating primitive cells are unable to obtain food for their growth In the developing eye the retina is of neural origin and the cells concerned are microglial in nature In the other coats of the eye the cells are *macrophage in type The migrating macrophages from the neural crest passing to* all tissues of the body would appear to be an essential link in the action of the chemical organizers of the embryo which they concentrate and bring to bear on their target cells in the developing and differentiating organs Thus reticulo endothelial *cells and the neural crest and its derivatives serve a truly trophic function in relation* to other embryonic cells

In the embryo the growth of nerve fibres into relationship with the macrophages of a tissue occurs at a later period than the macrophage migration to the same tissue *In certain circumstances for example benign congenital tumours the outgrowing* nerve fibres of the embryo fail to come into relationship with the macrophage syncytium of a tissue (see below)

In early development the neural crest cells migrate in close relationship to the *large vessels lying dorsally on either side of the notochord and many of these cells* remain in this perivascular position and migrate to various situations lying on the blood vessels They are seen as the perivascular melanoblasts and the chromaffin cell precursors thrown off on the tributaries of the cardinal veins of the embryo Their *derivatives appear in this perivascular situation in the adult for example* as the paraganglia In the earliest embryos macrophages from the neural crest come into close relationship with the walls of the developing large vessels and invade a differentiating tissue usually around the capillaries in the form of pericytes This is *very well seen in the developing central nervous system*

Any influence acting on the macrophages during embryonal development may alter the rate of or otherwise interfere with normal growth and give rise to some minor type of congenital abnormality of growth such as occurs in cases of rubella affecting the pregnant woman This may explain the occurrence of cleft palate in the offspring of mothers given cortisone in early pregnancy (Harris and Ross 1956)

During normal development many different cells exhibit attraction to one another resulting in migrations or a tendency to grow together This is seen in the attraction of growing ends of nerve fibres into relationship with dividing cells (Weiss 1955) and in the migration of neural crest cells and of the Wolffian body In the developing kidney the cells of the nephrogenic cord grow into contact with those of the ureteric bud the outgrowth of the Wolffian duct to form the secreting and collecting units of the kidney The twin primitive heart tubes and neural folds also attract one another and grow together Neighbouring cells and tissues often exert

while subject to exception is so generally true as to establish the great significance of chronic irritation as a factor in tumour genesis (Ewing) Thus warts may occur on eczematous skin or following herpes zoster (Wyburn Mason 1955) or herpes simplex (Wyburn Mason 1957b) Fibromata or angiomata may appear after zoster Lichen simplex of which pruritus vulvae is a variety lichen planus psoriasis and eczema including varicose eczema may be followed by malignant change to rodent ulcer or squamous carcinoma Malignant tumours of the skin may also follow herpes zoster (Wyburn Mason 1955) or herpes simplex (Wyburn Mason 1957a) In mucous and ulcerative colitis the inflammatory and ulcerative changes are present in the mucosa of the colon Polypi may form and show a tendency for malignant change to occur

The Kangri cancer develops on the abdomen of the Kashmir natives as a result of repeated irritation by the heat of the earthenware vessels which they carry next to the skin for warmth Epithelionata of the skin may appear in the areas of burns injury lupus vulgaris or osteomyelitic sinuses (Marjolin s ulcer) (see Hueper 1942) Frost bite of the ear or extremities which leads to inflammatory changes and hyperkeratosis may be followed by squamous carcinoma of the skin (Hueper) In the buccal mucosa malignancy may develop in an ulcer resulting from the chronic irritation caused by sharp teeth or as a result of repeated burns in clay pipe smokers when it is usually preceded by the chronic inflammatory change known as leuko plakia Leukoplakia in the pharynx and œsophagus or in the vagina also predis poses to the development of malignant change The site of predilection of œsophageal cancer is at the point of narrowing where contact with food and irritants is most intimate and prolonged The changes in the tissues in cases of gastric ulcer are those of a chronic inflammation and this predisposes to malignant change The mucosal scars on the stomach resulting from drinking of acids alkalies and other chemicals not infrequently turn malignant (see Hueper) Experimentally gastric carcinomata were produced in rats by feeding them on cockroaches infected with nematode worms which cause chronic irritation (Fibiger 1913) Malignant change in the bladder rectum or liver has followed the chronic irritation and inflammation resulting from the presence of hook worm ova Malignant growths of the gall bladder or pelvis of the kidney have arisen from chronic irritation of calculi A carcinoma of the uterine cervix not infrequently develops in an area of chronic cervicitis Carcinomata of the bronchial mucosa may appear in regions affected by chronic pulmonary tuberculosis or bronchiectasis or around foreign bodies (Siddons and Macarthur 1952)

Hormonal Influences on Tumour Formation

The uterus breast prostate and thyroid gland are organs whose activity is largely controlled by hormones and cyclical increases in their activity and growth followed by regression occur during life Administration of œstrogens produces hypertrophy of the breast thyroid and uterus Large doses or prolonged admini stration may be followed by the appearance of leiomyomata (fibroids) or carcinoma of the uterus adenomata or carcinoma of the breast adenomata of the thyroid or chromophobe adenomata of the pituitary gland (Gardner 1948)

PART III

THE ROLE OF MACROPHAGES IN TUMOURS

CHAPTER XIII

KNOWN AGENTS CONCERNED IN BENIGN AND MALIGNANT TUMOUR FORMATION

A NUMBER of agents are now known to predispose to the development of both benign and malignant tumours Thus prolonged exposure to the elements especially sun and ultra violet light favours papillomatous formation and the appearance of rodent ulcers and squamous carcinomata in the skin Chronic exposure to X rays may lead to benign tumour formation such as papillomata of the skin angioma lipomata fibromata etc to rodent ulcer formation or to malignant change which may affect any tissue The administration of arsenicals by any route may if long continued cause the appearance of papillomata or angiomata in the skin or to malignant change to rodent ulcer or squamous carcinoma In addition malignant growths of the internal and other organs may occur These include carcinoma of the tonsil œsophagus stomach colon kidney bladder prostate pancreas breast bronchus and sarcoma of the chest wall They are sometimes multiple (Sommers and McManus 1953) The inhalation of both nickel and chromium compounds may also result in the appearance of malignant growths of the nasal passages and bronchi (Hueper 1947) The entrance of such substances as phosphorus arsenic lead manganese copper alcohol chlorinated hydrocarbons coal tar etc into the liver may result in multiple nodular proliferations of the bile ducts and liver cells and adenomata of the liver and bile ducts Later cholangio carcinomata and hepato carcinomata may occur (see Hueper 1947)

Aniline dye workers are predisposed to papilloma and carcinoma of the bladder Smoking appears to favour the development of bronchial carcinoma while chronic exposure of the skin to tar or the carcinogenic agents derived from it leads to the appearance of warts or papillomata rodent ulcer or squamous carcinoma Chronic irritation by tar or its active constituents will produce experimental cancers in the oral cavity stomach and intestine When injected into the tissues extracts of tar may cause sarcomata or leukæmia (see Willis) Many azo compounds including dyes halogenated aliphatic hydrocarbons and urethane are also carcinogenic in the liver The filterable agent responsible for the Shope skin tumour in rabbits induces both benign papilloma formation and squamous carcinoma

Chronic irritation and inflammation may result both in benign tumour formation and also in malignant transformation The role of chronic irritation and its resultant inflammation in predisposing to the development of malignancy is generally accepted Billroth s dictum without chronic inflammation cancer does not exist

the testicle on one side This never subsided and afterwards gradually increased in size with the onset of malignant change Sarcomata of bone are known to develop *directly as a result of fracture in certain cases I have observed the appearance of* an osteosarcoma of the radius developing during the healing of a fracture sustained by a fall on the hockey field An osteosarcoma of the ribs of a dog followed the kick of a horse (Schulte and Welz 1952) and a bone sarcoma after uncomplicated healing of a fracture of the mandible due to a shot wound (Schmitt 1952) A number of well documented cases of traumatic carcinoma of intestine and colon are known (see Hueper 1949) Gruber (1923) records a case of abdominal crushing which was followed by the development of pyloric carcinoma Melchior (1929) another which was followed by carcinoma of the sigmoid colon Sauerbruch (1925) described a case in which a blow in the right flank was followed by hæmaturia and later by the appearance of a sarcoma of the right kidney Rueckart (1923) a case in which a blow on the left side of the trunk preceded the development of a hypernephroma and Wells and Cannon (1930) one in which a blow on the chest appeared to lead to carcinoma of the bronchus An epithelioma of the male urethra followed injury in the case described by Cooke (1952) and carcinoma of the penis similarly developed in Amelar s (1956) patient plasmocytoma (myeloma) of bone followed trauma in that of Schmauss (1952)

There is a small number of cases for which an important ætiological role of trauma in the subsequent development of glioma must be sustained Marburg (1936) recorded one in which a glioma originated from scar tissue in the brain of a man wounded five years previously the bullet remaining lodged in the brain Neuburger (1926) and Fischer Wasels (1932) described similar cases Merzbacher and Uyeda (1910) reported the history of a young man who immediately after a fall on his head developed the symptoms of a right sided skull fracture Some time after the accident epileptiform attacks occurred with increasing severity and frequency At death three and a half years after the injury two large tumours were found in the right brain at autopsy The two tumours differed in colour and consistence and one partly surrounded the other The more superficially located sarcoma originated from the pia arachnoid while the second tumour a glioma was situated beneath originating from the cerebral tissue The demarcation between the two tumours was sharp in places while there was a mutual penetration in other areas In addition to the tumour several foci of traumatic softening surrounded by a zone of glial tissue were found in the brain

Congenital Tumours

In many subjects benign lesions called tumours but which may well be termed congenital malformations are found at birth They consist of papillomata angiomata pigmented or vascular nævi lipomata fibromata etc They have the same structure as the similarly named tumours developing post natally They may undergo malignant change In some cases a malignant tumour is present at birth or develops soon after These are usually sarcomata Wilms tumours of the kidney ganglioneuromata or neuroblastomata (Wells 1940)

Trauma Preceding Tumour Formation

We have seen that local trauma however produced results in stimulation of phagocytic activity of the macrophages and this is followed by repair processes in which redifferentiation of multipotential cells takes place Following injury benign tumours or malformations may develop Faworsky (1932) describes the formation of neurofibromata after injury to the peripheral nerves In some subjects local trauma has given rise to an angioma (see Löwenthal 1895, Ewing 1940) The author has observed this change following a burn on the palm in a child of 7 years a scratch on the cheek in a child of 3 months a sprain of the ankle in a girl of 16 years, a blow on the back of the thumb with the starting handle of a car in a man of 33 years, and sitting on a knitting needle in a woman of 28 years Lipomata may also develop at sites of trauma or pressure such as that of a corset (see Buschke and Mattisohn 1914 Hueper 1942 Adair Pack and Farnor 1942) They may follow the injection of many different substances such as arsenic quinine iron or insulin into the subcutaneous tissues (Adair *et al* Sutton and Sutton 1939) On three occasions the author has observed cases in which a parotid swelling arose immediately after a severe blow over the gland This did not subside but later increased in size and subsequently proved to be a mixed salivary tumour

The role of trauma in the production of malignant change has been much discussed Owing to the constant exposure of the body to trauma which occurs without malignant change developing and the argument put forward that trauma only draws attention to a condition already present there are many who deny its role in precipitating malignant change in the tissues Statistical methods are often applied in attempts to assess the role of trauma in causing malignant change Ewing (1940) remarked that the relationship of trauma to cancer would be greatly simplified if the statistical tendency were replaced by clearer ideas of the results of trauma in different tissues and the mechanism by which such lesions lead to tumour growth Studies in this field are as meagre as the statistical contributions are superabundant As regards the skin however the role of trauma is generally accepted and books on dermatology contain many examples of this relationship This being so it seems inconsistent to deny its importance as a factor in causing malignant change in other tissues Sequeira (1957) mentions the case of a man in whom repeated striking of the face by the recoil of a rifle led to the development of a rodent ulcer in the area of the blow In a personally observed case a rodent ulcer developed in the skin subjected to pressure by the pad of a truss meant to contain an inguinal hernia I have collected numerous other examples of rodent ulcer and squamous carcinoma after trauma such as blows burns gun shot wounds etc from the records of the Royal Marsden (Cancer) Hospital

As regards the deeper tissues and viscera the relationship of trauma to malignant change is more difficult to prove Trauma to a lipoma may lead to malignant change as in the case described by Adair Pack and Farnor (1942) Cases in which tumours of the breast and testicle have developed directly as a result of a blow are well recognized and the latter are documented by Gordon Taylor (1948) The author saw one such case in which a blow by a cricket ball resulted in severe swelling of

of the testes and ovaries In seventeen species of tobacco plants pure genetically conditioned tumours have been observed in F_1 hybrids Such observations suggest that the predisposition to tumour formation is genetically controlled in some way

Viruses and tumour formation

In animals benign or malignant tumour formation may result from virus infection In man certain types of skin papillomata are due to viruses Mention has been made above of benign or malignant tumours which have followed herpes simplex or zoster lesions Whether viruses play any part in tumour formation in man remains to be seen

Conclusions

Benign or malignant tumour formation commonly follows chronic or acute inflammatory changes and tissue repair including those produced by trauma Certain carcinogenic factors which also produce inflammatory changes greatly predispose to tumour formation in the inflamed tissues Stimulation of growth of tissues by hormones may also give rise to tumours Tumours similar to those occurring postnatally may also appear in prenatal development and are then difficult to label as malformations or tumours In some cases genetic factors predispose to benign or malignant tumour formation *

* The various agents and factors concerned in tumour formation are discussed at length in Cowdry E V (19[illegible]) Cancer Cells Saunders Philadelphia

Heredo familial Tumours

In some families a tendency to develop benign or malignant tumours exists The conditions are inherited They include

(i) Multiple polyposis of the colon This disease is characterized by hyper trophy of the whole of the colonic mucosa and the presence of local areas of adeno matous thickening of the epithelium forming polypi Similar tumours are seen in some cases of Von Recklinghausen s disease The tumours tend to turn malignant

(ii) Peutz s syndrome Families are described in which there occur ephelides especially of the face and of the mucosæ including the mouth and there is multiple polyposis in the nose and nasal sinuses stomach bladder or intestine combined in various ways (Peutz 1921 Kisch cited by Meirowsky 1933 Crone and Light 1954 Walker Brash 1954) The multiple polyposis of the mucosæ is preceded by hypertrophy of the whole epithelial lining of the organs The tumours may turn malignant

(iii) Congenital cystic kidneys This condition may be unilateral or bilateral when it is often heredo familial Malignant growths are liable to develop in the affected kidneys

(iv) Von Recklinghausen s disease In this condition the cells of the various benign tumours and anomalies may undergo malignant transformation

(v) Retinoblastomata Apart from their association with congenital anomalies of the eye these growths may occur as an heredo familial disease The tumours appear soon after birth and usually in both eyes simultaneously or almost so

(vi) Tylosis (keratosis palmaris et plantaris) is an inheritable dominant charac teristic A family in which numerous cases of carcinoma of the œsophagus occurred in association with tylosis was described by Clarke Howel Evans and McConnell (1957)

Apart from the above the inheritance of a predisposition to cancer is occasionally seen in man Rarely a family such as those described by Warthin (1925) and Hauser and Weller (1936) and by Savage (1956) show so many members in successive generations with some kind of cancer that an inherited disposition cannot be doubted The familial occurrence of leukæmia is occasionally reported (see Carter 1957) That a liability to cancer is inherited was shown by Haldane (1938) who found that the chances of cancer appearing in an individual are much greater if one of his parents suffered from it Again uniovular twins are much more prone to tumours than are binovular while racial differences in proneness to tumour formation in different organs are known for example the observation that Japanese women are only one sixth as liable to breast cancer as compared with European women

There is a great deal of evidence from animal and plant sources indicating that the tendency to tumour formation is genetically controlled (see Huxley 1958) Thus guinea pigs appear to be non cancer prone while mice and budgerigars are Different strains of mice are liable to develop cancers of different organs By inbreeding the liability to cancer may be increased to 100 per cent All grey horses are extremely prone to malignant disease (chiefly melanomata) as compared with horses of other colours Generic crosses in birds are also very prone to develop cancer

of the testes and ovaries In seventeen species of tobacco plants pure genetically conditioned tumours have been observed in F_1 hybrids Such observations suggest that the predisposition to tumour formation is genetically controlled in some way

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CHAPTER IX

SOMATIC MUTATION CONGENITAL ANOMALIES AND BENIGN TUMOURS

Heteroplastic and Xenoplastic Transplantations

THE term heteroplastic usually refers to tissue combinations between different species of the same genus and xenoplastic to those between more distantly related forms Parts of embryos belonging to different species and genera may be substituted early in development The cells of the donor of necessity differ genetically from the host Rotmann (1939) employed two species of *Triturus cristatus* and *tæniatus* The former is larger and its eye vesicles correspondingly so He grafted gastrula ectoderm of *T cristatus* in place of the future eye region of the smaller *T tæniatus* embryo The eye vesicle later forming from the graft was usually of the same size as the normal host eye but in a few cases it was larger than that of its host This corresponds to the condition of unilateral buphthalmos in man In the reciprocal experiment *T tæniatus* ectoderm in the larger *T cristatus* formed a vesicle appropriate in size for the donor species that is much smaller than the normal host vesicle This corresponds to the condition of unilateral microphthalmos in man A balancer organ induced by head structures of *T cristatus* in transplanted prospective belly ectoderm of *T tæniatus* is a typical tæniatus balancer (Rotmann 1935) Harrison (1935) using embryos of *Ambystoma punctatum* and *tigrinum* which are animals of different sizes and different genetic make up carried out hetero plastic transplantations of the branchial neural crest on one side and obtained gill arches of different sizes on the two sides Again the larvæ of urodeles and anurans are genetically different and differ in various specific structures The urodeles are equipped with a pair of balancers on the ventro lateral side of the head and posse s dentine teeth whereas the anurans have a pair of suckers more ventrally and no dentine teeth but horny denticles There are also differences in the shape number and topographic relations of the visceral cartilages etc It was found that if appro priate tissue was transplanted early in development from a part of one embryo to a corresponding part in the other then the pattern and structural characters induced were those of the donor which differ genetically from the host and structures could be induced which do not occur in the genetic repertory of the inducing host (Holt freter and Hamburger 1955) Again if the neural crest is transplanted on one side then that half of the host embryo may develop donor pigmentation (see Hörstadius 1950) Clearly genetically different material may exist in an animal and produce structures abnormal in size and foreign to that animal

Many inherited bodily characteristics are dependent on the presence in the fertilized germ cell of specific genes If these genes change this is followed by change in the bodily characters In certain subjects multiple genetic mutations occur at the same time in the fertilized germ cell and a number of inherited anomalies may

be found together There is every reason to believe that genetic mutation is as frequent in somatic cells as in germ cells (Lockhart Mummery 1934 Burnet 1957) The essential difference between a mutation occurring in a germ cell and a similar process in a somatic cell depends simply on the extent and distribution of the cells descendent from that in which the mutation occurs and that the mutation is not inherited in the latter case A germ cell mutation can potentially influence all the body cells of all those individuals who receive the modified allele in question A somatic mutation can influence only those body cells in the individual organism which directly descend from the mutant cell

Somatic mutation has been studied particularly in plants and insects for example Drosophila but its occurrence in vertebrates and man has as yet received scant consideration Mutation occurs more frequently (some allege only) during cell division and in the course of repeated tissue subcultures of normal cells (Earle *et al* 1943 Sanford *et al* 1954) We have seen above that there is a constant repair process involving cell division and differentiation occurring in various tissues of the body throughout life It is possible that mutation may occur during this process The chances of such mutation increase with increase in the number of cell divisions for example in infancy and youth during repair processes or stimulation of growth and during physiological increase in growth activity The longer the animal lives the more the chance of somatic mutation occurring Mutations may thus appear theoretically at any time during life from the single cell to death including the period of intra uterine development and the genetically different material may remain in the tissue and grow as in cases of heteroplastic and xenoplastic transplantations

In man the conditions of neurofibromatosis iris pigmentation polydactyly syndactyly microphthalmos epicanthus albinism supernumerary teeth piebald hair and hare lip and cleft palate are each characters determined at least principally by a single main gene occurring in the fertilized ovum (see for example Ford 1942)

Unilateral Anomalies Associated with Congenital Heterochromia Iridis in Man

By the term heterochromia iridis we refer to a condition in which the irises of the two eyes are different in colour a state indicative of a difference in the genes in the pigment cells in the two eyes In some cases this may occur as a simple condition unassociated with other anomalies and sometimes inherited (Stern 1955) but rarely it is accompanied by unilateral developmental anomalies both in the eye and its surroundings (see Koby 1921) and also elsewhere in the body These are uninherited The anomalies of the eye affected and its surroundings include coloboma of the iris and choroid coloboma of the macula a persistent membrane in the anterior chamber and a white pellicle at the free edge of the iris melanosis oculi which is the counterpart of the cutaneous nævus marked by focal or diffuse excess of pigment cells in the choroid iris optic nerve sheath and conjunctiva and sometimes on the eyelids and over the skin of the face on one side unilateral melanosis oculi associated with a trigeminal vascular nævus (see Koby Mann 1937) unilateral buphthalmos (Lutz 1928) a facial hemihyperplasia with its corresponding difference in growth rates on the two sides buphthalmos and a facial vascular nævus

covered with neuro angio lipo fibromata (Case 2 Fig 71) a unilateral Sturges Weber syndrome that is a trigeminal and corresponding intracranial nævus often with buphthalmos (Weber 1929) I have observed a case of heterochromia iridis



Fig 70 Warty hyperkeratotic excrescences over congenital angioma associated with hetero chromia iridi Section of se eral of these showed them to be compo ed of neu o fibro l po angiomatous t ssue The la ge tumour was a teratoma The eye on the affected s de wa microphthalm c and a small meningocœle of the orbit was present Note the malformation of the e ir *The left was normal*

with a meningocœle and teratoma of the left orbit deformity of the ear and left microphthalmos (Fig 70) Heterochromia iridis has al o been recorded with uni lateral microphthalmos (Wal h 1947) with intracranial lipomata (Eckart 193) and syringomyelia (Walsh) Heterochromia iridis may be found in cases of Von Pecklinghausen s disease (Tannhauser 1926) and facial or body asymmetry (Scalinci 1915 Walsh 1947) I have observed a patient with a blue iris on the left and a brown on the right in which *the left half of the face and most of the front and sides*

of the neck showed areas of hyperpigmentation with patchy flat and raised nævus formations and these were also present in bands on the front of both arms and fore arms and irregularly on the left leg The left side of the base of the neck and the right face and part of the left frontal region showed areas of achromia The condition of partial albinism seen strikingly in negroes may be linear or metameric and may be accompanied by heterochromia iridis and piebald hair (Meirowsky 1933) (Fig 72)



Fig 1 Left fac al h m hyperplas a ass ted w th h t chromia d trig m nal nævus buphthalmo and c ngenital patent interventr cula septum (Ca ?) The e i much s ring from treatment

Differences in genetic material in the two irises are thus evidently accompanied by a tendency to growth anomalies in and around one eye and to disturbances in pigmentation in other parts of the body Among these are differences in the size of the eye vesicles We have seen above that transplantation of genetically different prospective eye material into early embryos may give rise to unilateral buphthalmos or microphthalmos When such conditions occur in man they presumably indicate that the cells composing the eye are also genetically different on the two sides Thus

covered with neuro angio lipo fibromata (Case 2 Fig 71) a unilateral Sturges Weber syndrome that is a trigeminal and corresponding intracranial nævus often with buphthalmos (Weber 1929) I have observed a case of heterochromia iridis



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Heterochromia iridis partial cataract atrophy of the bulb of the eye or phthisis bulbi may also be present (Walsh 1947)

In cases of general hemihypoplasia again every tissue is affected including the bones fat muscles joints limbs lymph nodes larynx thyroid kidneys the chambers of the corresponding side of the heart the blood vessels breast suprarenal gland ovary testis and parathyroids Narrowness of the palpebral fissure partial dental aplasia and defects of nails may be found on the affected side (Calnan 1949) and the hair growth and pigmentation may differ on the two sides It may be associated with *Ollier s disease or chondrodysplasia* (Zondek 1945)

In cases of facial hemihyperplasia the changes again roughly correspond to the trigeminal distribution (Fig 71) There is considerable increase in volume of the soft parts of one side of the face involving muscle subcutaneous tissue and fat The skin is congested and rugose and is often more pigmented than on the normal side The facial bones nasal orifice tissues of the cheek lips hard palate tonsil *tongue mucous glands of the cheek and salivary glands are notably larger than on* the other side The mucosa of the cheek is granular and the papillæ of the tongue larger and more numerous on the affected side The frontal and parietal bones in the affected area may show hyperostosis forming bosses Radiographs reveal an increased density of bone and changes identical with those of Paget s disease may be present and this may also be found histologically (cases 2 and 3) Leri (1926) described a case of facial *hemihyperplasia* in a child of 5½ years of age in whom the fontanelle closed prematurely and there was premature falling out of the temporary teeth and eruption of the permanent set on the affected side Moreover the calcification of the teeth germs was much advanced on this side as compared with those on the normal and with normal children This was also a feature of Case 3 The teeth may be larger on the affected side (Pushton 1937 1942 Miles 1944) (Case 2) The overgrowth of one side may be associated with a trigeminal vascular and pigmented nævus in the affected skin (see Case 2) The vascular nævus may be covered with hyperkeratotic skin or soft pedunculated warty growths which on section prove to be neuro angio lipo fibromata (Case 2) Exophthalmos buphthalmos or glaucoma may occur on this side (Bing 1939) Heterochromia iridis may be present with facial asymmetry (Scalinci 1915 Walsh 1947 Case 2) In two personally observed cases epicanthus was present only on the larger side (Wyburn Mason 1950)

In cases of general hemihyperplasia all the tissues on one side of the body are larger than on the other The skin may be thicker hyperkeratotic more highly pigmented vascular and hairy on this side (Gesell 1921 Cassirer and Hirschfeld 1935) The fatty deposits are larger the connective tissue thickened and buphthalmos cutaneous vascular segmental nævi phlebectasia and congenital varicose veins may be found on the hyperplastic side on which the hair may be darker and thicker The kidney tonsil suprarenal gland and testicle on the affected side are larger than on the normal Power may be greater and the temperature higher on the larger side In some recorded cases of hemihyperplasia such as those of Hornstein (1893) and Arnheim (1898) autopsy showed that the corresponding side of the heart both auricle and ventricle were enlarged and the blood vessels on this side were larger



Fig. 72 Congenital partial segmental albinism in a negro

like the differences in iris colour the growth anomalies and other lesions such as melanosis vascular nævi the teratoma lipomata angio lipo fibromata meningocœle syringomyelia and partial albinism associated with heterochromia iridis may arise from differences in the genetic make up of the anomalous material that is they are due to somatic mutation occurring during development

Congenital Hemihyperplasia and Hemihypoplasia (body asymmetry)

These congenital uninherited disturbances are to be distinguished from hemiatrophy and hemihypertrophy which are acquired conditions The congenital conditions may be largely confined to the trigeminal distribution or affect one half of the body In any given case it is often impossible to decide which is the normal side Facial or general hemihypoplasia or hemihyperplasia may complicate cases of syringomyelia and syringobulbia of Von Recklinghausen s disease or other congenital diseases of the central nervous system (Landauer 1939)

As already said the manifestations of facial hemihypoplasia are largely confined to the trigeminal distribution on one side The condition becomes more noticeable as the subject grows older There is a relative failure of development of all the tissues in the affected region including the skin subcutaneous tissues muscle and bone as compared with the other side The skin is thin pale and relatively unpigmented with little or no hair Sweat and sebaceous secretion is diminished and the fat less than on the normal side The muscle of the cheek the nasal cartilages and bone and jaws are smaller There may be an incomplete or delay in eruption of both the deciduous and permanent teeth in addition to other changes (Rushton 1944 Sprawson 1946–47) The bones are less dense Half of the tongue the mucosa of the cheek the mucous glands salivary glands tonsil and papillæ of the tongue are all smaller than on the normal side The pinna and eye may be smaller in size The superficial temporal artery may be smaller and pulsate less than on the normal side

numerary teeth and digits (polydactyly) syndactyly and epicanthus all of which are characters controlled by genes and not represented in the normal genetic make up of the individual For such unilateral changes to be present gene substitution would seem to have occurred on one side They are like the individuals which are obtained by heteroplastic and xenoplastic transplantation experiments and this suggests the cells on the affected side differ genetically from the normal In addition there may occur on this side various benign tumours and other anomalies such as lipomata angiomata fibromata localized disturbances in bone formation cryptorchidism etc Such observations appear to show that all such growth anomalies as are found in cases of hemihyperplasia and hemihypoplasia are related to differences in genetic make up between the abnormal and the normal tissue or of the tissues on the two sides of the body A parallel condition can be produced experimentally in animals

Von Recklinghausen s Disease

Von Recklinghausen s disease may occur as a heredo familial disease or as an isolated case in an otherwise normal family when it must arise by germ cell mutation The disturbances in the disease affect any part of the body and any tissue The commonest lesions are tumours in the skin and peripheral nerves and various types of nævus but many other growth anomalies also occur The skin lesions include pigmented areas varying in colour from yellowish to dark brown and in size from flecks to plaques or sheets They sometimes have a metameric distribution In other cases pigmented often hairy moles (nævus nervosus) or vascular nævi likewise sometimes having a metameric distribution may be present A trigeminal nævus is an example of the latter Pigmented vascular or depigmented anæmic nævi may also be found Facial or general hemihyperplasia or local gigantism of a limb heterochromia iridis (Tannhauser 1929) and buphthalmos may be present Fatty tumours of a metameric distribution are also recorded Eichenlaub (1921) and Sutton and Sutton (1939) described a linear nævus which histologically was a fibromyxoma Tumours form on the nerve roots and trunks and on the finest nerve branches Thousands may be present and affect not only cranial and spinal nerve roots and branches but are also found throughout the autonomic system in all viscera and mucosæ such as the alimentary tract from the mouth to the rectum and the bladder in bone and periosteum in the iris and in the various endocrine glands Skin tumours are pure fibromata In other cases the tumours are really neuromyomata or neurolipomata They always have a well marked angiomatous element which in some cases is so pronounced that they may be termed angio fibromata angiomyxomata or angiolipomata The tumours which are benign in the sense of pursuing slow development sometimes undergo sarcomatous trans formation especially those of large size and as a sequel to partial excision which may markedly stimulate growth Occasionally a general state of sarcomatosis may exist and the tumour growths everywhere become malignant at once Growths may also develop in tissues other than nerves and multiple heterogeneous tumours may be found In a case of recurrent fibromata of the peripheral nerves observed by the writer there also appeared recurrent fibro adenomata of both breasts and

than on the other exhibiting abnormal thickening of the *media and intima* The brain on the affected side is larger than on the normal Hemihyperplasia with precocious puberty was recorded by Harwood (1932) Heterochromia iridis may occur (Meirowsky 1933) In a personally observed case asymmetry of the two sides of the body was accompanied by heterochromia iridis and by two vascular nævi one at the nape of the neck and the other over the lower dorsal spine The number of teeth on the larger side may be greater than normal Polydactyly syndactyly or cryptorchidism may be present on the larger side or congenital heart disease and hypospadias may co exist The bone lesions known as Ollier s disease or unilateral chondrodysplasia may be associated with body asymmetry (Zondek 1945 Walsh 1947) This disease usually has a completely unilateral distribution The bones on the affected side become a different length from those on the normal and contain multiple areas of unossified cartilage in which the normal process of bone formation has not occurred In some cases areas of cutaneous pigmentation may be found in a distribution related to the osseous changes and in girls the disease may be associated with precocious puberty Cavernous angiomata and phlebectasia (Maffucci s syndrome) may also be present

In children there occasionally occurs an increased deposition of fat on one side giving rise to congenital hemi obesity (Hutchison 1904 Shaw 1914–15) This may occur with or without hemihyperplasia In Hutchison s case (Hutchison 1904) which came to autopsy suprarenal, kidney testis and half of the thymus on the *affected side were larger than on the normal but there was no increase in size of* the bones In congenital diffuse lipomatosis the lipomata may be unilateral They may occur alone (pseudo hypertrophy) but are usually associated with corresponding enlargement of the ipsilateral muscles and bones The latter show thickening and osteoporosis and may be co existent with diffuse cavernous hæmangiomata (see Von Winiwarter 1892 Adair Pack and Farrior 1942) The latter described two cases In one a boy aged 11 years the left arm and hand were larger than the right at birth and had grown more so since A diffuse lipoma was present over the left thenar eminence and also in the rest of the limb Radiographs showed deformity and enlargement of the bones of the thumb and index finger *The other case was a girl aged 6 years At the age of 1 year a small lipoma appeared on the posterior* aspect of the right shoulder and eventually involved the entire right upper limb The skin over it was roughened lymphoedematous and pigmented Between the *elbow and wrists was a large subcutaneous mass with keloid formation over it* X rays showed much enlargement and deformity of the right ulna and lower half of the humerus After operative removal large tender keloids formed Berenbruch (1890) described another case of hemihypertrophy with three congenital angiolipo mata over the right chest an angioma of the capsule of the right kidney and another of the cord substance and a thoracic extradural angioma on the right side connected with the angiolipoma on the back by a large plexus of veins

In animals *inherited* genetic asymmetries occasionally occur (Stern 1935) but the above asymmetries are not inherited In association with excessive growth of one side of the body or part of the body as compared with the other the larger side may exhibit a difference in iris colour hair colour and growth eye size super

the nature of the disease was reported by Crocker (1903) and already mentioned He described the case of a subject of Von Recklinghausen s disease who sustained a transverse lesion of the cord and this was followed by an enormous enlargement of parts below the lesion the skin hanging in large folds

The viscera may be involved Weise (1902) records a case of exclusively visceral localization of the disease with partial overgrowth of the stomach and duodenum Ford (1946) neurofibromatosis of the splanchnic nerves or pelvic plexuses with giant appendix megacolon or papillary adenomatosis of the intestinal mucosa and Feyrter (1948) the so called Rankenneuroma or disc like polypoid tumours of the gut of Von Recklinghausen s disease They are found in the small or large intestine or as a giant tumour (ganglioneuroma) in the appendix They consist essentially of a plexiform tumour like development of the local coarse fine or finest nerve network with overgrowth of the nervous ground tissue and reticulin fibres In the nervous networks are overgrowths of ganglion Schwann and endoperineural cells There is an overdevelopment of the mucosa of the gut covering the lesion and of the local muscle fibres In addition the fibres can be seen surrounding and lying within the walls of the blood vessels This vascular neurofibromatosis can occur in any or all layers of the vessel wall Kuhl (1952) describes a case of neurofibromatosis with a giant bladder This was due to the gigantism and hyperplasia of the nerve plexus in the wall The latter were neuromata and neurofibromata and there were marked vascular changes as described by Feyrter

The various tumours of the skin and other anomalies may be noticed at birth develop soon afterwards or appear at any time during life (Ormsby and Montgomery 1943 and others) Certain factors however influence their development They include puberty pregnancy intercurrent infections and lead poisoning Crocker (1903) mentions a case in which enormous folds of skin formed on the palms soles sides of the neck nose and tonsils (necessitating tonsillectomy) after an attack of scarlet fever Developmental anomalies of every conceivable type may also be present such as meningocœle unilateral hare lip and cleft palate buphthalmos bony defects of the skull cryptorchidism spina bifida syndactyly and polydactyly fusion of the vertebræ congenital heart disease and teratomata The writer has observed two cases in which supernumerary breasts existed In one case this was unilateral In another case recurring adamantinomata were present

Thus in this disease apart from the benign tumours including fibromata angiofibromata lipomata etc which are inherited and controlled by gene substitution in the germ cells there may occasionally be seen heterochromia iridis body asymmetry *unilateral* buphthalmos polydactyly syndactyly cleft palate and hare lip angiomata pigmented nævi supernumerary breasts and teeth teratomata cryptorchidism adamantinoma and other errors of growth but these are not inherited Now we have seen that iris colour syndactyly polydactyly cleft palate and hare lip changes in the eye size and supernumerary teeth are all conditions controlled by genes Since the changes are found unilaterally and are uninherited in this disease somatic mutation seems liable to occur during development and to be responsible for some of the associated unilateral and uninherited anomalies

recurrent fibromyomata of the uterus Malignant chromaffin tumours may be present in the adrenal medulla or organ of Zuckerkandl (see Willis) There may be meningiomata and nodules in the dura suggestive of fibroblastomata Innumerable *meningeal tumours may be found* Melanosis of the meninges with benign segmental skin melanomata is also described Angiomata of the central nervous system may be present Within the nervous system have also been found ependymomata astrocytomata hæmangiomata etc As many as five different types of meningiomata and gliomata have occurred in a single case Acoustic neurofibromata may form part of a generalized neurofibromatosis and may be associated with hyperplasia of the brain hypertrophy of the glial cells of the cortex endothelioma fibroma and psammoma of the dura and sarcoma of the brain Glioma of the optic nerve may be associated with symptoms of neurofibromatosis multiple fibromata of nerve trunks and gliomata of the brain (Ewing 1940) Ford (1946) described a case of plexiform neuroma of the orbital tissues and eyelid with buphthalmos and gliomata of the optic nerve and temporal lobe and another case with plexiform neuroma over the cranium defects in the skull bones and enlargement of the orbit Von Recklinghausen s disease is not infrequently associated with syringomyelia Lumbosacral tumours consisting of fatty fibrous myxomatous and angiomatous tissue or a dermoid or teratoma may be found partly within and partly outside the spinal canal and projecting through a spina bifida This is usually covered by areas of skin showing hypertrichosis and pigmentation In case I there developed a carcinoma of the rectum and a glioma of the cord Skeletal changes may also occur

Other manifestations of the disease are those known as plexiform neuroma and the related pachydermatocœle and elephantiasis neuromatosa When the whole distal fan of a nerve or of contiguous nerves is the seat of disturbance changes are found in the tissues supplied by the nerve The skin and subcutaneous tissues are involved in a diffuse fibrous swelling which feels soft and in which coiled convoluted or beaded nerve cords can be distinguished Over it the skin is sometimes pigmented or nævoid development occurs This disturbance may be present alone but often accompanies other signs of Von Recklinghausen s disease It may affect any part of the body but especially the head face and neck Sometimes one side of the tongue or the lips are involved producing a hemihyperplasia In some cases the condition known as pachydermatocœle develops and the proliferated skin and subcutaneous tissue hang down in folds often of enormous size In others the tissues of the growth are even more voluminous and result in a great overgrowth of a part such as the whole of one limb or of the scalp In any of these varieties hyperostosis of bone in the vicinity is not uncommon The bones of the limb may be greatly enlarged bowed rough and pitted and in some cases longer than their fellows When the tissues are examined microscopically they are found to contain enormous numbers of overgrown nerve fibres which in many cases are unmyelinated or finely myelinated (Spittel and Fernando 1929) Within their meshes much fibrous tissue is present and angiomatous change may be found The skin may be pigmented In some cases a local gigantism of a digit or part of a limb occurs without a local neurofibroma An observation which throws considerable light on

In all benign tumours of epithelia (papillomata) the basement membranes are intact and macrophages lie in them and between the epithelial cells but are unaccompanied by nerve fibres If there is an angiomatous development below the epithelium the capillaries are dilated and contain macrophages in their walls but no nerve fibres are present Often in the pedicle of a benign tumour the blood vessels are normally differentiated the capillaries possessing pericytes and unmyelinated nerve fibres may be found In such cases the tissues of the pedicle do not form an essential part of the tumour

(1) *Lipomata* A macrophage syncytium like that of normal fatty tissue is present in lipomata but no nerve fibres The tumours thus consist of mutant fat cells growing without inhibitory nervous control to their macrophage syncytium

(2) *Neurofibromata and Fibromata* My investigations with vital dyes have shown that the tumours consist of fibre producing non staining fibroblasts interspersed with spindle like vitally staining macrophages The former are presumably mutants and it is therefore not surprising that according to Willis (1948) the collagen of neurofibromata stains differently from normal Myelinated nerve fibres often traverse neurofibromata but no unmyelinated fibres supply the macrophages At the margins of neurofibromata the macrophages and fibroblasts of the growths are continuous with the endo perineural cells The tumour tissue is not well circumscribed nor encapsulated but mingles with the surrounding neural and other tissues

(3) *Congenital Melanomata of the Skin* (pigmented nævi) It has been shown that melanomata probably arise by somatic mutation and that the pigment cells are genetically different from the normal cells of the body By using vital methylene blue staining pigmented nævi can be divided into two groups (*a*) pigmented flat nævi in which nævus cells are present in the corium beneath the pigmented epidermis and which show no evidence of thickening or overgrowth of the epidermis hair follicles skin glands or angioma formation and in which the nerve supply of the tissue is intact Macrophages or melanophores which have taken up melanin are present in relation to the melanoblasts and the clear and Langerhans cells of the skin and the Schwann cells of the nerve fibres are also deeply pigmented (*b*) Hyperkeratotic raised pigmented nævi often with hypertrophy of hair follicles and skin glands and angiomatous formation beneath the epidermis In these Langerhans and clear cells of the epidermis stain normally and macrophages are present in the vessel walls but no nerve fibres are found in the thickened epidermis or angiomatous vessel walls A bundle or bundles of nerve fibres can be found to one side of the skin lesion The presence of Langerhans and clear cells in the epidermis in these lesions was also described by Masson (1926) The mutant nævus cells accompanied by melanin containing and vitally staining macrophages may or may not be found in the corium in these nævi These are considered further below Graduations from (*a*) to (*b*) also exist Masson also described an abnormal thickened leash of nerve fibres in such anomalies and regarded the nævi as neuro nævi My findings suggested that the leash of nerve fibres did not actually enter the pigmented lesion and that they appeared deflected from it

Conclusions on the Mutant Nature of some Congenital Anomalies and Benign Tumours

From a consideration of the disturbances of growth associated with hetero chromia iridis hemihyperplasia and hypoplasia and with Von Recklinghausen s disease and the experimental evidence it would appear that —

(1) In association with heterochromia iridis the associated disturbances including buphthalmos microphthalmos vascular nævi melanosis oculi pigmented nævi angio lipo fibromata lipomata teratomata congenital anomalies of the eye and meningocœle are due to somatic mutation during development and the material of the lesions is genetically different from normal

(2) In cases of body asymmetry the lesions on the larger side such as buphthal mos pigmented nævi vascular nævi angiomata lipomata angio lipo fibromata syndactyly polydactyly cryptorchidism hare lip supernumerary teeth and breasts epicanthus disturbed bone formation and syringomyelia and congenital heart disease are probably the result of somatic mutation and the material of the lesions probably differs genetically from that on the normal side

(3) In Von Recklinghausen s disease again unilateral buphthalmos polydactyly syndactyly cleft palate hare lip angiomata supernumerary teeth and breasts teratomata cryptorchidism and adamantinoma and congenital heart disease may occur and appear to be due to somatic mutation

(4) While the various benign tumours fibromata angiofibromata pigmented nævi and lipomata in Von Recklinghausen s disease are inherited and the result of gene substitution in the germ cell these same tumours occur in unilateral conditions and in association with heterochromia iridis and are then presumably due to somatic mutation

It seems probable that *many uninherited congenital growth anomalies and all benign tumours such as fibromata angiomata and lipomata are due to somatic mutations occurring some time in early development* and they consist of material genetically different from the rest of the body When there is a great disparity in the size of paired organs the effect is comparable to that resulting from the trans plantation experiments described in which the organs on the two sides differ geneti cally As seen from these experiments genetically different cells may exist in the body from an early date without giving rise to inflammatory or immune reactions which are responsible for graft incompatibility in adults Willier and Rawles (1940 1944) and Dagg Karnofsky Toolan and Roddy (1953) basing their work on the observations of Burnet Stone and Edney (1950) and Billingham Brent and Medawar (1953) showed that if genetically unrelated cells are introduced sufficiently early into embryonic tissues they are not recognized as foreign in later immuno logical development They may thus persist unattacked by *immune reactions into* adult life In so far as benign tumours developing later in life and singly appear to be identical in structure and behaviour with congenital tumours they are also presumably derived from mutant cells

Some Features of Benign Tumours and Malformations

The occurrence of vitally staining cells and the absence of nerve fibres in all benign tumours (apart from flat benign melanomata) have already been discussed

(1) Nævus telangiectoides and nævus flammeus in which the thin walled vessels especially the capillaries are dilated but empty on pressure

(2) Nævus angiomatosus (angioma cavernosum) in which the abnormally dilated thin walled vessels will not empty on pressure

(D) Nævus lipomatodes for example the case described by Robinson and Ellis (1937) These consist essentially of lipomatous formations often combined with angiomatous elements

(E) Angioma verrucosum in which a warty or verrucose and often a lipomatous angiomatous nævus are combined Sometimes fibromatous nodules are present

A and B have already been considered The latter consist of a general over growth of the layers of the skin with hypertrophy of hair follicles and skin glands and sometimes with nævus cells in the corium Macrophages are present throughout the lesion but no nerve fibres though abnormal leashes of these may be found to one side

Vascular nævi consist of an enormous dilatation of the capillaries in the affected area Macrophages are present in their walls but no nerve fibres are found The overlying skin is frequently thickened and keratomatous and every gradation from this condition to pigmented hyperkeratotic hairy nævus may occur The skin may be studded with irregular warty excrescences (nævus verrucosus) (Fig 70) Some pigmented or vascular nævi may contain much soft connective tissue and fat (nævus lipomatodes) (Ormsby and Montgomery 1943 Ewing 1940) Between the vessels there is usually an increased deposition of connective tissue and of fat (angiolipoma) A vascular nævus may also be associated with considerable new growth of connective tissue described under the name nævus vascularis mollusciformis (see Ewing 1940) When a limb is the seat of an extensive angioma there is often a pan hypertrophy of the tissues of the limb When the nævus involves the trunk the breast on the affected side may be different in size from that on the normal (Wyburn Mason 1950) In a personally observed case red marks were present on the left ear at birth The c gradually increased in size spread to the neck and occipital region and on to the face The hæmangioma was accompanied by subcutaneous extensions more extensive than the skin discolouration When seen at the age of 3 years there was obvious hypertrophy of the left ear Iacchia (1929) reports the case of a patient with a nævus in the mandibular division of the trigeminal area passing on to the neck which developed after birth It was associated with hyperostosis of the mandible and hypertrophy of the tongue on this side

Pigmented vascular nævi are not infrequently segmental linear or unilateral in distribution or confined to the territory of a peripheral nerve or sensory dermatome The best known examples of such conditions are the pigmented nævus nervosus lateralis and the vascular trigeminal nævus but such segmental nævi may recur in any part of the body S[l] in lesions may extend in long bands corresponding to segmental root areas (nævus verrucosus) The changes in association with a trigeminal nævus may affect the mucosæ of the cheek palate no e and tongue The affected side of the skull may be larger than the normal There is often a local facial hemihyperplasia involving all the tissues including the ear In other

An observation of some significance is that a flat pigmented nævus may be surrounded by an area of hypochromic or achromic skin (Rolleston 1910 Sutton 1916 Weber 1929), in which the melanoblasts are deficient

The mutant nævus cells in the dermis have been thought to have dropped *down from the basal layers of the epidermis a theory supported by Dawson (1925)* and others Masson (1926) believed that nævus cells arise partly from the cells of Schwann partly from the cellules claires and partly from cells of the special touch corpuscles in the papillæ of the corium He stressed the occurrence of local neurofibromatous change Feyrter *(1938) recognizing the objections to Masson s* hypothesis that Schwann cells are the source of nævus cells supposed the latter to come from Masson s clear cells or Langerhans cells in the epidermis and he proposes for such nævi the term neuro endotheliomas However all pigment cells reach the epidermis by migration from the neural crest The observation that the skin around a flat melanoma may show an absence or deficiency of melanoblast cells may mean that in fact the flat melanomata result from an *error of migration of the primitive mutant melanoblasts* into the basal layers of the epidermis Rather than dropping down from the epidermis into the corium as Dawson suggested the cells would seem in fact to be in process of *passing into* the epidermis in excessive numbers When the nævus is surrounded by an achromic area it may be that the mutant melanoblasts which should have migrated into the achromic area have been diverted to the pigmented area where they are in excess Partial or complete albinism is explicable on a failure of migration of mutant non pigment producing melanoblasts to the skin and eye either locally or completely The occasional segmental distribution of a flat pigmented nævus or of albinism is presumably due to a mutation in the cells of a single segment of a neural crest Raised hyperkeratotic pigmented nævi *will be considered below*

(4) *Melanosis oculi* the counterpart of the cutaneous nævus is marked by focal or diffuse excess of pigment cells in the choroid iris optic nerve sheath and conjunctiva Pigmented spots may also be present in the eyelids and over the skin of the face The changes may be associated with heterochromia iridis (Koby 1921 Mann 1937) The abnormal cells seem to be somatic mutants and this condition presumably results from their abnormal migration and failure to reach their destination completely and in normal amounts

(5) *Vascular Pigmented and Anæmic Achromic Nævi* Vascular nævi are of various kinds and may be divided into those exhibiting excessive pigmentation and overgrowth of tissues (raised hypertrophic pigmented and vascular nævi) and those showing the opposite changes (atrophic and anæmic type) Such pigmented hypertrophic and vascular nævi may be classified as follows —

(A) *Pigmented flat nævi without hyperkeratosis lipomatous or angiomatous* formations and with nævus cells in the corium (considered above)

(B) Warty nævus (nævus verrucosus) or raised hyperkeratotic pigmented nævi consisting of areas of hyperkeratosis or papillomata usually pigmented and *often* with overgrowth of hair and skin glands Sometimes nævus cells may also be found in the corium (see above)

(C) Vascular nævi

(8) *Adenomata of the Thyroid* Such tumours tend to arise especially in the hypertrophied glands occurring in goitre regions and in conditions in which the growth of the gland has been stimulated The tumours contain macrophages around the acini but no nerve fibres

(9) *Polypi of the Gastro intestinal Tract* These occur singly but also as heredo familial conditions both in multiple polyposis of the colon and in Peutz s syndrome The tumours presumably consist of mutant cells As shown such lesions contain macrophages but no nerve fibres

(10) *Mixed Salivary Gland Tumours* This normally benign tumour consists essentially of glandular acini atypical glandular acini with marginal epithelial sprouting solid glandular epithelial formations masses with cystic spaces or of epithelial filaments and networks The gland cells contain mucus droplets The formations lie in lakes of mucinous material mixed with collagen This gives a peculiar and variable metachromatic stain which differs from that within epithelial cells and the lumina of epithelial cavities (Kux 1931 Hemplemann and Womach 1942) The stromal connective tissue of the gland and the mucinous tissue mingle intimately

Investigations of these tumours with methylene blue and trypan blue showed that many cells lying in the mucinous matrix are macrophages and not epithelial cells Others are found in the capsule of the glands and the macrophages of the mucinous matrix mingle imperceptibly with those of the connective tissue of the gland and the capsule of the tumours Sheldon (1943) regarded them as myo epithelial in nature and such cells appear to be macrophages No nerve fibres are present in the tumour tissue The tumour presumably arises through cell mutation possibly of the acinar cells

(11) *Adamantinomata* The normal enamel organ consists of a downgrowth of buccal epithelial cells containing a vitally staining stellate reticulum This is normally attracted to comes into contact with and induces the formation of the dental germs or papillæ formed by the odontoblasts Adamantinomata may occur in the jaws and pituitary region and are often associated with congenital anomalies of the teeth They may also be found in cases of Von Recklinghausen s disease which suggests they are malformations and due to somatic mutation They consist of an overgrowth of enamel organ epithelium which gives rise to cell masses and clumps the centre of which is an open network with spaces consisting of vitally staining cells like the stellate reticulum of the enamel organ In other parts no stellate reticulum is seen but areas like basal cell carcinoma are found No evidence of dental papillæ are seen No nerve fibres were found in the columns or in close relation to them

Thus this malformation consists essentially of an enamel organ epithelium with its contained macrophages and presumably composed of mutant cells This fails to come into contact with or induce a dental papilla which normally influences its own differentiation or to attract the growing ends of nerve fibres

(12) *Teratomata* Teratomata are commonly found in association with Von Recklinghausen s disease and in combination with various other congenital abnormalities in the adjacent organs I have mentioned the case of teratomata of the orbit

cases of trigeminal nævus the eye may be enlarged (buphthalmos) (see Ballantyne 1930) and glaucomatous and the angioma may extend to the underlying meninges and even the corresponding side of the brain may be involved The cortex may be calcified and the hemisphere on this side enlarged in size as compared with its fellow The changes in the skull bones are those described as craniostenosis They consist of an overgrowth but a premature fusion of the sutures on this side (Ellis 1945-6) Such a segmental trigeminal nævus may be found in association with syringobulbia As stated above a trigeminal vascular nævus may be accompanied by facial hemihyperplasia Leri (1926) gives details of a case in which were present a hyperkeratotic linear vascular nævus in the left C8-D1 segmental areas and a greatly hypertrophied cervical rib in this segment on the same side only

Sometimes the grossly dilated vessels are found not only in the subcutaneous tissue but in the whole metamere extending deeply into the muscles and involving the corresponding vertebra and half of the cord (Klippel and Weil 1922 Schröpl 1927 Turner and Kernohan 1941 Wyburn Mason 1943) Karschner Rand and Reeves (1939) described a patient in whom a segmental vascular nævus of the skin was associated with a cavernous hæmangioma of the corresponding vertebral body Johnston (1938) records a case in which a cutaneous vascular nævus corresponded segmentally with an extradural cavernous hæmangioma and Kaplan (1395) one with a segmental nævus corresponding to an extradural hæmangioblastoma

The lesions would seem to be ascribable to somatic mutation at some early period in development The frequent excess of melanoblasts and the abnormal leashes of nerve fibres found in the hyperkeratotic lesions the latter apparently deflected from their normal course will be recalled They are evidence of failure of attraction of the growing ends of nerve fibres and of excessive attraction of pigment cells in relation to the mutant cells In cases of hyperkeratotic nævi affecting a whole body segment mutation presumably involves a cell or cells which produces this segment

(6) *Leiomyomata (Fibroids)* These consist of plain muscle fibres surrounded by reticulin and collagen fibres and by macrophages No nerve fibres are present Macrophages (pericytes) are found around blood vessels and are continuous with the macrophages between the muscle fibres

(7) *Fibro adenomata of the Breast* As described above although the existence of nerve fibres in relation to the normal breast acini has been denied I was repeatedly able to demonstrate their existence in both breast and prostate Each acinus is surrounded by a nervous network with Schwann cells attached These lie close to the basement membrane of the acini and ducts with their macrophage syncytium and basket cells between the acinar and duct cells In fibro adenomata of the breast however there has occurred a proliferation of reticulin and collagen fibres around the acini and the nerve fibres are divided from the basement membrane of the acini and ducts by a variable distance *Macrophages lying between the* fibroblasts in the fibrous tissue take up vital dyes No nerve fibres are found in this tissue Presumably the primary cause of this state is a mutation in the peri acinar fibroblasts

spina bifida (Johnson 1857 Von Recklinghausen 1886 Borst 1888 Cramer 1913 Small 1955)

There is often a dermal sinus passing downwards towards the defect in the vertebral arch and perhaps ending at the tip of the cord This is surrounded by pigmented telangiectatic and often hyperkeratotic skin which may be hairy Alternatively pigment may be deficient The subcutaneous tissue may exhibit angiomatous or lipo angiomatous changes The sinus may be of variable depth If it is shallow it is often continued to the arachnoid as a fibrous band the membrana reuniens posterior (Von Recklinghausen 1886) The line of this prolongation may be interrupted by one or more dermoids or epidermoids The arachnoid may be pulled out to form a meningocœle Surrounding the track of the penetrating dermal sinus or often at its bottom or connecting it with the arachnoid is a mass of tissue forming a congenital tumour This may extend into the meninges The tumour tissue may be a (neuro)fibroma myxoma angioma or cystic lymphangioma and often contains smooth striated muscle cartilage bone or glandular structures (like salivary glands) and may be teratomatous The tumours are not encapsulated and they may mix with the neural tissues of the cord or with some of the roots of the cauda equina Except in the case of teratomata however no nerve fibres are present in the tumour tissue itself The lesions presumably arise by somatic mutation towards the end of the laying down of the mesenchymal tissues by the primitive streak

(14) *Cryptorchidism* Failure or imperfect descent of the testes may occur unilaterally in Von Recklinghausen s disease or body asymmetry Not infrequently it is associated with a teratomatous formation In a case described by Whitehorn (1954) unilateral cryptorchidism was associated with complete unilateral Wolffian duct agenesis It may be supposed that the condition is due to somatic mutation in some of the constituent cells of the Wolffian body

(15) *Congenital Cystic Kidney* Developmentally the kidney is formed of two parts (1) a secretory portion the nephrogenic cord arising from the hinder end of the nephridial system and forming the cortex of the kidney the glomeruli convoluted tubules and the loops of Henle and (2) an ureteric bud which develops as an evagination of the hinder end of the Wolffian duct and forms the ureter pelvis and collecting tubules The two portions exert mutual inductive influences on one another If the nephric tubules fail to join the collecting tubules the condition of congenital cystic kidney arises This anomaly may be associated with many others and like them would seem to result usually from a somatic mutation though occasionally the genetic abnormality is an heredo familial condition

(16) *Syringomyelia* This condition is frequently associated with hemihyperplasia and with the genetically determined Von Recklinghausen s disease tuberose sclerosis and Von Hippel s disease with nævi and numerous other congenital anomalies It may be familial Like other anomalies in the above conditions it possibly arises by somatic mutation in the early ovum possibly of one half of the body so that the neural folds on the two sides consist of cells of different genetic make up The anomaly may thus result from an error of fusion of the genetically different material

associated with meningocœle microphthalmos and heterochromia iridis Like other congenital anomalies the lesions presumably result from somatic mutation at some time during development Testicular teratomata may be associated with incomplete descent of the organ (Willis) A teratoma of the bladder may be accompanied by atresia of the anus and urethra (Martini 1874) They occur over congenital defects of the lumbo sacral region when they are often associated with an angioma pigmented hairy nævus spina bifida or meningocœle and other abnormalities of the lower half of the body (see Ingraham 1944) A teratoma close to the cervical spine was accompanied by cervical spina bifida and congenital anomalies of the cord (Gerlach 1894 Henneberg and Koch 1923) Intracranial teratomata may be associated with cranium bifidum anencephalus palatal fissures etc A cervical teratoma was described in association with reduplication of the lower jaw (Wilms 1895)

Teratomata are mixed tumours which may include in their structure tissues derived from all three primitive germinal layers There is an infinite variety in the arrangement of tissues which may appear to have some order or consist of undifferentiated cells deficient in orderly arrangement and in which malignancy may later supervene The tissues may vary from those of a dermoid cyst with hair teeth etc *that is with little more than ectodermal derivatives to complex tumours* containing every type of tissue The primary sex organs (gonads) however are never present Pigmentation due to melanin producing cells is found in various tissues Nervous tissue is present in at least four fifths of all teratomata (Willis 1936) Peripheral nervous tissue is found as nerve ganglia of sympathetic type nerve bundles usually accompanied by plentiful Schwann and perineural cells and usually unmyelinated but sometimes myelinated and occasional well defined Pacinian corpuscles (Kaboth 1924 Hausmann and Berne 1932 Willis 1936 Nicholson 1937 Masten 1940) The nerves of these tumours are not derived from the host except that the skin of a dermoid may receive such fibres (Nicholson 1937) The tissues of the growth however do not contain the myriads of fine unmyelinated fibres of normal tissues In all its tissues such as perichondrium periosteum epidermis hair teeth connective tissue and fat macrophages are found as in normal tissues The enamel organ *of the teeth has a plentiful stellate reticulum* which is of course composed of reticulo endothelial cells

The structure of a teratoma varies with its position Thus central nervous tissue may be abundant in intracranial teratomata gastric and pancreatic tissue *in thoracic* and upper abdominal teratomata digits or limbs in sacro coccygeal and retroperitoneal teratomata that of the branchial arch contains rudiments of the ear The last are also associated with deformities of the tongue palate and jaw (Wallmann 1859) Such observations suggest that when some teratomata develop mutant multipotential cells which would normally pass to certain areas have been displaced and pulled out of position and so produce distorted structures in an abnormal position and at the same time the structures which should be formed by the original cell fail to develop properly at their normal site

(13) *Congenital Lumbo sacral Tumours* Congenital tumours are commonly found in relationship with *congenital lesions of the lower end of the spine* such as

of the cells of malignant tumours is their ability to feed without the aid of macro phages It may be that the cells of benign tumours also possess this property to some extent

Benign tumours show certain features which suggest they consist of cells whose metabolism is partly controlled by macrophages uninhibited by nerve fibres so that the normal method of diminishing the feeding of their cells does not exist

(1) Benign tumours show an excessive tendency to become inflamed

(2) Benign tumours show an increased metabolism as compared with the normal for example benign breast tumours have a greater uptake of P^3 than normal tissue (Tobin and Moore 1942 Das Gupta *et al* 1956)

(3) The cells of all benign tumours continually produce excessive amounts of their specific products as compared with normal tissue Thus papillomata or adenomata of the gut secrete excessive mucin and adenomata of endocrine glands for example thyroid islet cells or pituitary excess normal secretions

(4) As with normal tissues many benign tumours such as warts fibromata and neurofibromata are stimulated to grow by local irritation mild trauma or local incomplete attempts at excision Neurofibromata increase in size with local irritation pregnancy and various intoxications such as lead poisoning or intercurrent illnesses (Wilson 1940) In contrast with normally innervated tissues the tumours do not tend to decrease in size after the irritation has been removed All such factors stimulate the activity of macrophages throughout the body including those in the tumour It seems that rate of growth of a benign tumour is proportional to the phagocytic activity of its contained macrophages

(5) During wasting of the body the feeding of the body cells by macrophages is presumably inhibited by nervous discharge Benign growths are to some extent independent of general body metabolism Many lipomata continue to grow or remain unaffected during loss of body fat in phthisis and other wasting diseases (Virchow 1862 Madelung 1888 and others)

It is suggested therefore that the *essential basis of benign tumour tissue is the presence of mutant cells surrounded by macrophages which largely control their meta bolism but which themselves are uncontrolled and uninhibited by nerve fibres*

The Nature of Von Recklinghausen s Disease

Certain facts are worthy of note in considering the nature of Von Recklinghausen s disease

(1) The cells of the various tumours and malformations appear to be mutants and the raised pigmented and vascular nævi and the various tumours such as fibro mata myxomata or lipomata often with an angiomatous element contain no nerve fibres Any other benign tumour may develop None contain nerve fibres and they exhibit features indicating an absence of innervation of these tissues

(2) Crocker s case in which transection of the cord caused a local overgrowth of the tissues supplied by the lower half of the cord indicates the great importance of nervous impulses in controlling growth and suggests that normally centrifugal impulses descend the cord and inhibit the growth processes in the tissues below

(3) In cases of pachydermatocœle or elephantiasis neuromatosa the overgrown

(17) *Congenital Disease of the Heart* Major developmental anomalies of the heart may occur in cases of Von Recklinghausen s disease in association with asymmetry of the two halves of the body or with numerous other congenital anomalies such as syringomyelia or congenital cystic disease of the kidney Congenital malformations of the heart may also exhibit a familial incidence (McKeown *et al* 1953) In such cases presumably the tissue composing the whole or part of the heart differs genetically from the rest of the body

Conclusions as to the Possible Nature of some Congenital Anomalies and Benign Tumours

During normal embryonic development and repair processes various cells and tissues attract one another and may later exert mutual inductive influences (see Willier Weiss and Hamburger 1955) This is seen in the attraction of the ends of growing nerve fibres into relationship with dividing cells in the migration of neuroblasts and melanoblasts from the neural crest and the ingrowth of the latter into the epidermis and eye (choroid and iris) in the migration of the Wolffian body in the enamel organ which grows into relationship with the dental germ the two causing mutual induction to take place in the twin heart tubes which grow into relationship with subsequent secondary fusions and foldings in the kidney where the nephrogenic cord comes into contact with the ureteric bud the outgrowth of the Wolffian duct to form the secreting and collecting units of the kidney We have seen that these various attractions including those of nerve endings depend primarily on genetic considerations which control reactions akin to antigen antibody reactions (Tyler 1955) All benign tumours such as fibromata lipomata and angiomata and hypertrophic nævi do not contain nerve fibres while in the last named a leash or leashes of nerve fibres which have deviated from their normal destination are observable Julius (1929) studying tar papillomata in mice found the nerves deflected back at the edge of the tumour It seems that the *mutant cells of benign tumours fail to attract ingrowing nerve endings as do normal cells* The mutant pigment cells of flat pigmented nævi and of the tissues affected by melanosis coli fail to migrate normally from the neural crest to their objectives The mutant cells of hyperkeratotic nævi may attract melanoblasts abnormally while the cells of undescended testes consist of tissues which have failed to migrate normally Adamantinomata are composed of enamel organs without dental germs the two primordia of the teeth having failed to establish contact Benign teratomata consist of multipotential cells which have failed to migrate to their normal destination Polycystic kidneys result from failure of the two parts of the kidney to approach and fuse normally syringomyelia from failure of the neural folds to fuse properly some kinds of congenital heart lesions from failure of normal fusion of the twin heart tubes All of these conditions seem to result from somatic mutation during development It appears that *when somatic mutation occurs in the body cells at any stage of development the normal mutual attractions and inductive influences between the normal and mutant cells are disturbed* giving rise to lesions containing mutant cells and macrophages but often no nerve fibres and when this is so growing with their macrophages uncontrolled It will be seen later that an essential feature

accessory adrenal The melanophores were very widely distributed throughout the brain sheathing the larger vessels lining the endothelium of capillaries and forming rosettes Sweet and Connerty (1941) described the case of a new born infant with what was considered a malignant melanoma There were widespread darkly pigmented indurated elevated cutaneous tissues on the head trunk and peripheries the largest of which was covered by hair A confluent grey brown lesion covered the napkin region and extended down the right leg to the knee The external genitalia were replaced by a large fungating growth and similar masses arose from perineal and perianal regions The infant lived 17 days during which time the colour deepened perceptibly At autopsy were found numerous non pigmented nodules of tumour tissue in the liver masses of tumour cells containing pigment in the pons cerebral cortex and subcutaneous tissue and tumour masses arising from the perineal region The structure of some of the perineal lesions in which nerve fibres were present raised the question of a malignant melanoma developing from a congenital nævus or neurofibromatosis complicated by neurogenic sarcoma and congenital nævi A somewhat similar case was reported by Wilcox (1939) under the title melanomatosis of the skin and central nervous system because of the association of a large nævus that covered the skin from the upper portion of the thighs almost to the costal margin with melanin filled nævus cells in the brain and meninges Willis (1948) described a case of melanosis of the pia of the base of the brain and especially the frontal lobes Patches of slight brown pigmentation were present in the skin of the right side of the face and temporal area outer surface of the sclera of the right eye and slightly of the left A malignant melanoma of the meninges was present at the medial border of the right cerebral hemisphere

It will be recalled that all the melanoblasts of the body are derived from the neural crest from which they migrate to the skin eye structures and other parts of the body This migration takes place at the same time as that of the macrophages which tend to retain a perivascular and perineural situation and which form some of the cells of the meninges and endo perineural tissues and the satellite and Schwann cells and microglia and pass into the adrenal cortex and medulla and spleen It is thus significant that mutant melanoblasts may be found in certain circumstances in the body in abnormal situations chiefly perivascular on the peripheral nerves and sympathetic chain in the meninges perivascularly in the brain in the adrenal medulla spleen in the gut wall and gall bladder It suggests that such phenomena result from errors of migration from the neural crest of the primitive mutant melanoblasts which are perhaps dragged with the migrating macrophages also derived from the neural crest and settle down with the latter in abnormal situations

(B) *Teratomata* Teratomata occur especially in the testis ovary and broad ligament The affected testis may be undescended or ectopic or the testicular teratoma associated with pseudo hermaphroditism The teratoma may occur in the epididymis or spermatic cord They also occur in other situations These include

(a) The anterior mediastinum especially between the heart and root of the lung Here they may be closely connected with the great vessels at the base of the heart and project into the pericardial cavity

tissues contain myriads of unmyelinated nerve fibres and tissue overgrowth occurs in the areas where these nerve fibres are found The skin may show pigmentation overgrowth or a vascular naevus The deeper tissues exhibit a general increase in size excessive formation of connective tissue vasodilatation or an angiomatous formation and hyperostosis of bone There is in fact an overgrowth of mutant tissues but nerve fibres seem to fail to find the peripheral structures they are normally attracted to

The disease thus shows par excellence, the effects of mutation in the germ cells and in somatic cells giving rise to both inherited tumours and non inheritable growth anomalies The developing mutant cells of both lesions fail to attract the growing ends of nerve fibres which are normally inhibitory to the macrophages of the mutant tissues and this leads to aimless outgrowths of nerve fibres The gene complex in this disease appears to be unstable

Developmental Disturbances (Congenital Anomalies) and Tumours resulting from Disturbances of Migration of Neural Crest Cells

The macrophage cells originating in the neural crest of the embryo normally migrate to all tissues especially along the vessels The active macrophages stimulate the growth and differentiation of the tissues The melanoblasts likewise arise from the neural crest and normally pass to the eye and skin During their migration from the neural crest it seems that owing to disturbances of mutual attraction between themselves and mutant cells the macrophages may drag either mutant melanoblasts or multipotential cells no longer attracted to their definitive situations in the embryo by normal genetically dependent forces from the neighbourhood of the primitive streak and lateral side of the developing neural fold and notochord to abnormal situations There they settle down and produce pigment or lesions containing diverse tissues (teratomata) particularly in relation to blood vessels and nerve plexuses The reasons for these suggestions will be adduced from various sources

(A) *Extradermal Melanomata* Melanoblasts are occasionally found in the peripheral nerves and sympathetic chain in the spleen adrenal intestine and gall bladder They have been reported in the kidney in fishes They may also be found in the meninges optic sheath and on the cerebral (pial) vessels In some patients a considerable increase of these cells producing definite pigment spots and streaks has been observed repeatedly In Crahl s (1906) case an extensive pigmentary naevus of the bathing trunk area was associated with profuse pigment spots of the pia mater and Oberndorfer (1909) saw a similar extensive cutaneous naevus with symmetrical pigment spots and tumours of the cerebral pia Thorel (1907) observed diffuse pigmentation of the pia of the brain and upper cord which merged into a tumour process only at the cauda Some of the tumours have been multiple others single In Boit s case (1907) a tumour arose from the outer side of the spinal dura in Berblinger s (1915) an infant of nine months multiple melanomata of the skin and neurofibromatosis were associated with a melanotic tumour of the hippocampal gyrus multiple pigmented spots in various portions of the brain glioma of the pons diffuse perithelial sarcoma of the cerebro spinal pia with secondary deposits of pigment and a true

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(a) The anterior mediastinum especially between the heart and root of the lung Here they may be closely connected with the great vessels at the base of the heart and project into the pericardial cavity

(b) Retroperitoneally where they are found especially on the aorta or close to the superior mesenteric and cœliac arteries and behind the pancreas and kidneys A teratoma of the abdominal cavity attached only to the wall of the renal vessels was described by Potter (1952) Another has been de scribed attached to the upper pole of the left kidney and supplied by the renal and lumbar arteries Others have occurred in the kidney Such retroperitoneal teratomata may extend forward to occupy the root of the mesentery or mesocolon

(c) Behind the rectum and above the levator ani muscles in presacral and pre coccygeal positions These lie in front of the sacrum and coccyx The tumour is often attached to the periosteum of the anterior surfaces of the *lower sacral or coccygeal vertebræ The sacrum may be rudimentary* The spinal dura is rarely involved Hypertrophied sacral arteries and the branches of the sacral nerves course over the tumour which is attached to these structures

(d) In the neck anterior to the cervical viscera and in connection with the branchial clefts or within the thyroid gland In several cases the structure of the tumour has suggested branchial arch involvement such as when there are deformities of the tongue palate jaw and ear and the tumour contains rudiments of ear (Wallmann 1859)

(e) Attached to the roof of the nasopharynx or palate or with an intracranial portion connected by an isthmus traversing the sphenoid bone These may be composed of glial and brain tissue and associated with extensive deformities of the skull for example anencephalus hemi crania palatal fissures etc In other cases teratomata or dermoids are found in the hypophyseal or hypothalamic regions

(f) Multiple teratomata of the pia of the cord and brain have been recorded Potter (1952) reports a fœtus with no less than five teratomata within the brain substance

(g) Teratomata may occur anywhere along the dorso median line of closure of the *medullary tube from the third ventricle to the filum terminale* (Saxer 1896 Trachtenberg 1898 White and Tripp 1900) They may be found in the pineal gland or choroid plexus of the third ventricle or over the dorsal surface of the sacrum and coccyx where they are adherent to or *enclosed within the periosteum or connected to the bone by a pedicle*

Teratomata occurring in relation to the central nervous system are often associated with developmental defects of the latter and of the skull and vertebræ Thus a teratoma of the cervical region was associated with syringomyelia and central gliosis of the cord *in cases recorded by Gerlach* (1894) and Henneberg and Koch (1923) Those in relation to the lower part of the spine are often accompanied by sacral spina bifida (Vaton 1895 Témoin 1892 Lindsey 1896 Clutton 1898 Schwinn 1910–11 Barthelmy *1912 Park 1912–13 Broca 1913 Bucy and Haymond* 1932 Hermanns 1935)

(h) In the breast orbit subcutaneous tissues mesentery or omentum

Teratomata occur especially in the ovaries and testes hence the suggestion that they arise in some way from the totipotent germ cells by a process of partheno genesis Nicholson (1950) points out that in teratomata (of the ovary) in which there appears to be evidence of attempts to form a vertebral column the appear ances are deceptive and that histological examination shows that its bones are not discrete elements products of metameric segmentation but centres of ossification in one and the same cartilage and that the notochord is absent at points where it should be present in a vertebral column Again teratomata do not contain the primary sex organs (gonads) This is evidence that the object never underwent development on somatic lines or gastrulation and never possessed a primitive streak that is it has never developed from a totipotent sex cell by parthenogenesis The cell from which a teratoma originates is thus nearly but not entirely totipotent On the other hand in malignant teratomata of the testis there occurs tissue which looks like a chorion epithelioma and very frequently there is an excretion of anterior pituitary like hormone which gives a positive Aschheim Zondek test The metastases may have a similar structure This suggests a true totipotentiality and that in the case of the testis these tumours arise by mutation and a process of parthenogenesis

However teratomata may also occur in the epididymis and spermatic cord and in the broad ligament where sex cells are not found and in fact in any part of the body where totipotent cells do not occur normally In these cases no excretion of anterior pituitary like hormone occurs in the urine and extra testicular tumours appear to be of a different origin Certain of the observations quoted above are of significance in pointing to their nature

(1) Teratomata may occur attached only to an artery wall such as the renal by a stalk or in relation to the great vessels at the base of the heart and root of the lung or on the aorta or the sacral artery It is along the vessels of the embryo that macrophages and other neural crest cells migrate from the neural crest

(2) They occur on the pia arachnoid which is largely derived from neural crest macrophages

(3) They occur in close relationship to the central nervous system in the roof of the nasopharynx ventral to the vertebral column and over the site of the dorso median line of closure of the medullary tube in tissues derived from the neural crest region of the embryo and corresponding to the line of closure and fusion of the neural folds

(4) They occur *within* the brain especially at the base Macrophages (microglia) migrate to enter the central nervous system in this region

It seems therefore that teratomata occur at sites and along lines to which neural crest cells including macrophages migrate These sites in fact correspond to those where melanoblasts may also be found in abnormal circumstances Such observations suggest that when some teratomata develop mutant multipotential cells which would normally pass to certain areas have been misplaced or pulled out of position and so produce distorted structures in an abnormal position and at the same time structures fail to form properly at their normal sites

It might be that the essential disturbance in teratomatous formation (other

than in the testis) is that during migration neural crest macrophages drag with them mutant multipotential cells to abnormal situations where they continue to differentiate These mutant multipotential cells may originate close to the neural tube and thus have a wide potential or from more peripheral regions where their potential is more limited Their deviation from their normal destination will then lead to *developmental defects with which they are so commonly associated* While the multipotential cells continue to be surrounded and their growth controlled by macrophages the tumours remain benign If the macrophages disappear malignant change has occurred (see below)

(C) *Heterologous Meningeal Tumours and Tumours of the Posterior Nerve Root Ganglia Peripheral Nerves and Autonomic System* The meninges like the endo perineurium with which they are continuous receive cells from the neural crest removal of which causes local developmental anomalies especially in the lepto meninges Harrison (1938) points out that identical tumours may occur in the meninges posterior nerve root ganglia peripheral nerves and sympathetic The tumours which may develop from the leptomeninges include meningiomata of fibroblastic xanthomatous and myxomatous types and angioblastomata melano mata lipomata osteoblastic tumours and teratomata Those on the peripheral nerve roots include neurofibromata and melanomata (see Cushing and Weed 1915 Oberling 1922) hæmangioblastomata (endothelioma) (Wyburn Mason 1943) and tumours containing mixtures of lipomatous and hæmangioblastomatous tissue (Small 1935) They presumably develop from mutant cells

The microglial cells originate from the leptomeninges and so also from the neural crest The migration of microglial cells into the nervous system occurs especially in the inter peduncular fossa and the choroid plexus region of the third and fourth ventricles and at the site of attachment of the posterior nerve roots It is in these regions that there occur various tumours of non nervous elements identical with those of the meninges and also including lipomata angiomata hæmangioblasto mata dermoid cysts cholesteatomata and teratomata In the cord angiomata may occupy only one side Multiple lipomata may occur in the choroid plexus the base of the brain and spine They may be associated with defects of the skull malformed irides heterochromia iridis (Eckart 1935) absence of kidney hare lip and cleft palate (Baker and Adams 1936)

As with teratomata and melanomata such heterologous growths in the peripheral nerves meninges and brain can readily be explained by the dragging of mutant cells of multiple or abnormal developmental potentials by the migrating neural crest macrophages

CHAPTER X

THE MUTANT LIKE NATURE OF MALIGNANT CELLS THE FACTORS STIMULATING THEIR GROWTH AND THE SUGGESTED NATURE OF MALIGNANT GROWTHS*

Some malignant tumours can be transplanted into other animals of the same species and will continue to grow This was first shown by Jensen (1903) in mice At first this was only found possible in animals of the same breed but later they could be transferred to any species of mouse The operation required the transplant of tumour cells and transmission is only a grafting process as the tumour cells do not come from the new host The new host merely acts as a culture medium The successful transplantation of tumours depends on the stroma reaction of the host Toolan (1953) has recently accomplished autologous and homologous transplanting of cultures of human cancers into human volunteers

Malignant disease does not arise merely through loss of restraining influences of the stroma on the growth of normal cells This is shown by a number of observations

(1) Pure cultures of cells *in vitro* have clearly been removed from all restraints exercised by other tissues but cultures of normal cells do not grow into tumours when implanted into homologous animals while cultures of malignant cells of the same type produce tumours when implanted

(2) The unimpaired invasiveness of malignant tumours when transplanted from one animal to another shows that this cannot depend on any hypothetical loss of tissue restraints peculiar to the first animal

(3) Cells of primary growths continue to invade neighbouring tissues to depths far beyond the extent of stromal changes due to the carcinogenic stimuli which evoked the growth

Malignant cells appear to differ from the normal cells of the host The reasons for this statement are numerous They are based on the immunological studies of Pressman and Korngold (1953) Korngold (1956) and Rapport and Graf (1955) and chromosomal studies of Levan (1956) and certain arguments such as follow —

(*a*) When transferred to other sites and tissues of the body by metastases or by transplanting accidentally during surgical operations cancerous cells continue to grow and display undiminished invasive properties which are not displayed by grafts of normal cells experimentally transferred to the same site

(*b*) Many pure cell lines for example fibroblasts maintained in tissue culture while at first not so can become malignant when reinjected into mice (Earle *et al* 1943 Sanford Likely and Earle 1954) This change can be induced by chemical carcinogens

(*c*) Tumours of identical origin show widely different invasive powers and the particular degree of invasiveness displayed by a given tumour reappears repeatedly

* The mutant nature of malignant cells is discussed at length in The Physiopathology of Cancer (1953) Edited by Homburger F and Fishman W H Hoeber New York by Greenstein J P (1954) Biochemistry of Cancer second edition Academic Press Inc New York and by Koller P C in Cancer (19[illegible]) Edited by Raven R W Butterworth London

in its metastases and in the case of transplantable tumours in repeated transplants Each tumour maintains its own peculiar infiltrative and metastatic properties

(*d*) *Southam Moore and Rhoads* (1957) transplanted cultures of human cancer and normal cells to normal and cancerous patients They reported that cultures of malignant cells caused little reaction in cancerous patients but a violent reaction in the normal Homologous normal cells do not grow in normal or cancerous patients or *provoke a particularly violent reaction*

(*e*) Various factors are known to act as carcinogenic agents These include exposure to X rays and ultra violet light arsenicals nickel and chromium compounds tar and its contained carcinogens 3 amino 1 naphthol in the case of the bladder mucosa azo compounds and urethane in the case of the liver and the Shope skin papilloma factor in the case of the rabbit skin Carcinogenic agents have no general or direct action in stimulating the growth or metabolism of tissue culture cells and *many actually inhibit the growth and metabolism of cells in vitro* (*Earle et al* 1943 Haddow 1938 1944) However all these agents produce not only malignant but also benign tumours which seem to consist of mutant cells suggesting malignant cells may also be of this nature

(*f*) Congenital abnormalities such as benign melanomata of the skin melanosis oculi undescended or imperfectly descended testes teratoma syringomyelia polycystic kidneys and Ollier s disease of bone seem to be evidence of mutation in *the mother cells of the tissue It has often been observed that such developmental* errors are especially liable to undergo malignant transformation of their constituent tissues Renal carcinoma tends to develop in polycystic kidneys in adults and sarcomata in infants Wilms tumour may arise in kidneys showing microscopic foci of undifferentiated tissue forming congenital anomalies Hogan and Simons (1957) describe a renal adenocarcinoma in a child with reduplicated collecting system and an ectopic ureter opening into the urethra Cordonnier and Spjut (1957) record a *transitional cell carcinoma in an* extrophic bladder Doherty (1927) found that 27% of cases of melanosis oculi developed malignant melanoma A glioma may develop from syringomyelic cysts Retinoblastomata may be associated with *developmental anomalies of the eye such as microphthalmos or persistent embryonal* vessels or plexiform neuroma of the orbit and skull Development anomalies also appear to be more liable to malignant change This is seen in malignant change in sequestered portions of the breast and supernumerary breasts aberrant portions of the thyroid thymus pancreas uterus ovary and adrenal Kaplan (1935) recorded a segmental naevus associated with an extradural hæmangioblastoma

Devic and Tolot (1906) described a patient with body asymmetry There was a *congenital enlargement* of the left upper limb in which the superficial veins were enormously developed Later severe intermittent pain developed in the arm and with it an increase in size of the ipsilateral breast On the back over the left scapula there *appeared a lipoma* The breast was found at autopsy to have undergone complete angiomatous transformation and this change extended to neighbouring tissues and towards the axilla An angioma was present in the mediastinum *In* addition there were an*giomata of the tissues of the left loin* with excess of cellular

fatty tissue around the left kidney and multiple angiomata of the liver and spleen Roth (1938) in a female infant of 11 months with marked overgrowth of the left side of the body found a large embryonic tumour Since the cells of congenital anomalies and those in the enlarged half of the body in cases of hemihyperplasia are probably mutants it seems that the cells which develop malignant properties also differ genetically from the other cells of the body

(g) In the fish the Mexican top minnow (*Platypœcilus*) a single sex linked gene (Sp) merely produces dominant spotting The hybrids between this and the sword tail a member of another genus (*Xiphophorus*) presumably differing genetically are quite healthy unless they receive the gene Sp from the *Platypœcilus* parent which in the hybrid gene complex gives rise to a fatal cancerous melanomatous growth (Kosswig 1929) This suggests that malignant tissue differs genetically from normal

(h) In Von Recklinghausen s disease the cells of the various benign tumours and anomalies which have been shown to be mutants may undergo malignant trans formation

(i) The evidence that cancer proneness depends on genetic factors has been briefly referred to in Chapter VIII

To conclude such observations would appear to show that *the cells of malignant tissue like those of benign tumours are genetically and probably antigenically different from the cells of the rest of the body* They do not appear to give rise to normal antibody responses in the organism However powerful mutagens do not cause cancer when tested on mice (Berenblum and Shubik 1949) The invasive properties of tumours must reside largely in the tumour cells themselves though this does not mean that the host s tissues play no part The mutation like change has characters unlike other mutations and resembles that from damage to the self producing areas of the cell (Berenblum 1954) It has been shown that malignant tissues consist of cell masses in which are neither macrophages nor controlling nerve fibres Thus *an essential property of the cells of malignant tumours is that unlike normal cells they are able to a large extent to grow without the aid of macrophages to feed them or remove their metabolites*

All populations of cancer cells are heterogeneous and subject to the processes of mutation and selective survival Because of the latter a tumour strain can extend its virulence to other hosts and alter its response to agents being tested for thera peutic possibilities (Puck *et al* 1956 Rhoads 1957 Burnet 1957) Fisher and Holloman (1951) assumed that only one mutational step is necessary in the change of normal tissue to a malignant tissue but that cancer does not develop unless a number of mutated cells find themselves in juxtaposition If one mutational change occurs in random fashion throughout life and overt malignancy develops only when seven potentially cancerous cells are contiguous then the observed age incidence of cancer would be expected Nordling (1953) subscribed to the theory that multiple mutations are necessary for malignant invasion Stocks (1953) on statistical grounds suggested the number might be five Armitage and Doll (1954) arguing from the statistics of increased frequency of cancer with age concluded that six to seven mutations are necessary for malignant changes from normal tissue but later

(Armitage and Doll 1957) found acceptable a two stage theory of cancer When a benign changes to a malignant tumour it may be that either further mutation like changes occur or only that the bodily control of the mutant cells is completely lost during this change

Benign or malignant tumour formation may occur during prenatal development or any time after birth In many subjects a liability to develop mutants seems to be due to a genetic instability in cells Mutants may appear in response to known carcinogenic agents to trauma or to chronic inflammation in all of which the cells of the affected region are stimulated to grow and divide and during this process mutant cells may appear Mutation may also occur simply during cell division in the normal repair process occurring all through life in the ovum fœtus or after birth In cases in which a carcinogen plays a part in the appearance of a mutant several possibilities as to its mode of action exist

(1) It may cause mutation by direct damage to chromosomal material in random fashion Those mutations which release the control against excessive growth will then prosper

(2) It may merely stimulate regeneration and increase the chances of mutation occurring

(3) It may act on some key cellular component render it non functional and induce all of its descendants to undergo a genetic change

As yet we cannot answer these questions It may be that both (2) and (3) are operative

The Significance of Macrophages around Malignant Cells

Single or scattered mutant malignant cells may rest in the tissues for years and show no tendency to multiply (Willis 1952 Hadfield 1954) A frequent finding at autopsy in cases of carcinomatosis is the presence of many tumour cell emboli which have evoked a surrounding inflammatory reaction and are in the process of dissolution (Willis 1941 1952) Similarly one not infrequently observes a patient who develops distant metastases 15–40 years after removal of the primary growth (Hadfield 1954) It is not uncommon to find similar cell masses in the spleen (Jaffe 1938) Apparently tumour cells can be restricted in a dormant state in the tissues for many years Such tissues have of course a normal macrophage content controlled by nerve fibres

Malignant growths are usually surrounded by a wall of macrophages lymphocytes plasma cells and occasionally polymorphs This is a highly significant feature of malignant tissue growth There are many reasons to suppose that this wall of macrophages tends to restrain malignant tumour growth

(1) The role of macrophages around tumours has been investigated by many observers (Fichera 1910–11 Borghi 1926 Favilli 1930 Bogomoletz 1936 see Jaffe 1938 Franceschini 1955) Borghi (1926) reported that during the transplanting of tumours in animals there are hypertrophy and hyperplasia of the reticulo endothelial and macrophage cells that this is especially marked in animals in which the tumour development is slow and that it is slight in cases where tumour develop

ment is rapid Numerous observations showing that the degree of local collections of macrophages associated with fibrosis parallels the resistance to the growth of malignant tumours are forthcoming (see Franceschini 1955)

(2) The spleen is an important antibody producing organ of the reticulo endothelial system Malignant metastases are rare in the spleen though small groups of malignant cells may sometimes be found there (Jaffe)

(3) Foulds (1932) using Brown Pearce rabbit tumour found transplants gave metastases frequently in adrenals kidneys and eyes and less often in liver and lungs Intravenous injections gave similar dissemination In animals vitally stained by repeated injection of trypan blue that is in which the activity of the reticulo endo thelial cells was altered the spleen liver and lungs were now the commonest sites of metastases Evidently vital staining with its subsequent alteration of macro phage activity had diminished the normal reactive infertility of these organs as sites for metastases

(4) In experiments in which the reticulo endothelial system has been blocked with dyes etc the animals showed a lowered resistance to the spread of malignant growths (see Jaffe 1938 Franceschini 1955) Ludford (1931) found that trans plantable tumours grew more rapidly in mice vitally stained with trypan blue Kavetzki and Diadjuscha (1937) reported that stimulation of the reticulo-endothelial system lowered the incidence of development of carcinomatous metastases in rabbits inoculated intravenously with Brown Pearce carcinoma whereas blockage of the system raised the incidence of tumour growth Diadjuscha (1937) has also claimed that implantation of Ehrlich s carcinoma into mouse liver or spleen or of Flexner Jobling carcinoma into rat testis was invariably successful when the reticulo-endo thelial system was blocked and rarely so when it was stimulated Bogomoletz (1936) suggested that the functional state of the reticulo endothelial system decisively conditions the resistance of an individual to cancer

(5) X irradiation first stimulates and then depresses the activity of macrophages Vorlander (quoted by Zachert 1930) tested the effects of X irradiation of different intensity on the resistance of mice to tumour grafts He found that weak dosages made animals more resistant and high dosage more susceptible Krebs (1943) adapted a lymphosarcoma by inoculating mice after X irradiation Once grown in the immunologically weakened host it became capable of transplantation to the normal growing animal previously resistant to it Poussy and Guerin (1947) also found that transplantation of sarcomata into rats is more easily done and growth is greater if carried out after successive doses of X rays (see also Glucksmann 1950 Kaae 1953 Kaplan and Murphy 1949 Mottram 1937 1938 Von Essen and Kaplan 1952) Large volume irradiation increases the risk of metastases in animals even if the primary is regressing Tavares and Morais (1937) found that in mice pretreatment with thorotrast favoured the growth of Ehrlich carcinoma

The importance of pretreatment with X rays in causing growth of tumours is illustrated by the following case reported by Cohen (1956) A 59 year old man presented with a 6 cm diameter squamous carcinoma of the dorsum of the left hand of two years duration This was treated with superficial radio therapy Seven months later there was an obvious local recurrence and involvement of the epi

trochlear and axillary lymph nodes All three sites showed squamous carcinoma on biopsy and were treated by intensive irradiation For the succeeding six months the patient was well except for a small necrotic ulcer at the primary site He then suddenly developed a febrile constitutional reaction with a generalized macular rash The skin rash faded within a few days except for those lesions inside the irradiated areas which persisted and increased Some weeks later each macule within the irradiated skin fields had *developed into a palpable tumour The lesions* became confluent forming two rectangular tumour masses exactly demarcating both axillary treatment fields Biopsy of these lesions showed unpigmented malignant melanomata Although the primary melanoma was not found the patient dying shortly afterwards without necropsy there was no doubt that wide spread melanoma cell embolization had occurred but that all tumour emboli were effectively suppressed except in those tissues subject to irradiation Schurch (1934) saw a similar case of nodular metastases of gastric carcinoma in an irradiated area of skin

(6) Not only large doses of X rays but also cortisone depress macrophage function Toolan (1954) has recently grown cultures of human cancers in hamsters previously treated with X irradiation or cortisone Toolan (1957) also succeeded in growing embryonic gut and stomach in the external chest wall of adult homo logous animals previously treated with cortisone Baserga and Shubik (1954) found that cortisone favoured an increased metastatic spread of all experimental tumours studied

(7) Local trauma also primarily affects macrophage activity which may be increased or decreased It often alters the rate of growth of tumours (Deelman 1922 1924 Des Ligneris 1940 Friedwald and Rous 1944 1950 1951 Lacas sagne 1933 Mackenzie and Rous 1941 Pullinger 1940 1943 1945 Rous and Kidd 1941) Thus attempts at incomplete surgical removal of malignant growths may stimulate the rate of growth of the tumour Cases where metastases grow more rapidly in injured or inflamed tissues are described (De Craene 1911 Firket 1925 Willis 1930 Dahlin and Henderson 1956) Such observations relate the rate of growth of tumours to changes in the reticulo endothelial cell activity

Thus it appears that all factors which affect the activity of macrophages around malignant cells may also affect the rate of growth and of metastatic spread of a cancer Any factor which depresses the activity of macrophages favours the growth of tumours and vice versa Now the basic reason for failure of transplanted tumours to grow in an heterogeneous strain has been claimed to be the immune response of the new host to foreign marker antigens in the graft (Burnet 1957) If these are lost the tumour develops a progressively wider range of transplantibility Loss of marker antigens may be a primary determinant of malignancy If this is so the effect of macrophages on tumour growth may be accounted for by the fact that they contain and concentrate immune bodies round the growth and engulf tumour cells

Suggestions as to the Nature of Malignant Tumours

A malignant tumour has been defined as a new growth of tissue growing apparently spontaneously possessing an atypical structure subserving no use and with no definite termination of its growth largely or wholly uncontrolled by the

organism and growing at its expense Willis definition is that a malignant tumour is an abnormal mass of tissue the growth of which exceeds and is unco ordinated with that of normal tissue and persists in the same excessive manner after cessation of the stimulus which evoked the change (Willis 1948) Nicholson (1933) states that failure of co ordination is *the* distinctive character of every malignant tumour Cancer is a breakdown in the control of cells and the co ordination of their activity with that of other cells of the body Yet in spite of many allegations to the contrary the cells of malignant growths differ from normal and are mutants The failure in cancer is due not to any weakness of the organism but to a change in the character of the cells rendering them in one way or another insusceptible to the normal control (Burnet 1957) A cardinal point of Armitage and Doll s theory is that only if a mutation results in the loss of some structure or function by which general control over growth is exercised will the descendants of the mutated cell become sufficiently numerous to produce clinically or chemically detectable results

In the foregoing pages it has been deduced that the reticulo endothelial cells concentrate circulating food stuffs hormones vitamins and other substances and present them to normal tissue cells They thus bring hormones to bear on their target cells They also receive the waste products of cells In this way they control the metabolism and growth of normal cells The activity of the reticulo endothelial cells is inhibited by impulses passing to them in the unmyelinated nerve fibres In this way the reticulo endothelial system is an essential link by which the metabolism of the cells of a tissue is controlled and correlated with the rest of the body by nervous and hormonal mechanisms Stimulation of macrophages is responsible for the phenomena of inflammation and is essential for the processes of regeneration of a tissue Macrophages are concerned in the full differentiation of cells in the embryo It has been seen that normally various cells including macrophages are attracted to other cells of a tissue through auto antibody or immune body phenomena which are dependent on genic actions

A growing malignant tumour tissue consists of masses of mutant cells apparently genetically different from normal and capable of growth and division without the normal feeding by macrophages Within the mass are found neither macrophages nor functioning nerve fibres but around it are collections of macrophages A feature of the tissues of a malignant tumour appears to be that the mutant cells do not attract macrophage cells or nerve endings as do normal cells Such attractions have been seen to depend on auto immune and genetic processes The mutant cells of a malignant tumour do not appear to excite normal auto immunological pheno mena The absence of inflammatory responses the failure of regeneration after injury and perhaps to some extent the tendency to necrosis in the tissues of a malig nant tumour can be related to this absence of macrophages

Single or small collections of mutant malignant cells may exist in the tissues for long periods surrounded by macrophages and nerve fibres and show no tendency to unrestrained growth In rare cases notably in neuroblastomata and retinoblasto mata spontaneous slowing or even cessation of growth and regression takes place in malignant tumours (see Willis 1948) Neuroblastomata then change their charac ter to that of ganglioneuromata This is accompanied by the appearance within

the growth of Schwann cells and macrophages and by differentiation of the neuroblasts of the growth to form ganglion cells and axons with applied Schwann cells. In such cases the entry of macrophages and Schwann cells (which have trophic functions in relation to other cells) has to some extent restored the normal control of mutant cells which are now more differentiated. It seems that the absence of macrophages and nerve fibres could be an essential feature of malignant tumours and a manifestation of the loss of control of the metabolism of its cells. The wall of macrophages round a malignant tumour is perhaps evidence of an attempt to re-establish macrophage control of the mutant tissue.*

Factors Precipitating Malignant Invasion by Mutant Cells

(A) *Those associated with known Carcinogenic Agents acting on previously Normal Tissues.* Reference has been made above to the uptake of particulate or chemical carcinogenic agents applied to a tissue by the reticulo-endothelial cells and also to the fact that both tar compounds and X-rays applied to a tissue lead firstly to stimulation and later depression of the phagocytic activity of its contained macrophages. Brief mention has been made of the known factors which may induce benign and malignant tumour formation, that is cell mutation in previously normal tissues. These same factors also produce other phenomena. In all occupational skin cancers the onset of the tumour formation is preceded by a chronic dermatitis, hyperkeratosis, folliculitis and acne. Exposure to the elements likewise leads to inflammatory changes in the skin and hyperkeratosis. Chronic exposure to X-rays is followed by inflammatory changes in the skin and other tissues and to hyperkeratoses and the irradiated skin is sensitive to light. Again arsenicals cause hyperkeratoses and, when ingested or injected, lead to inflammatory changes in the skin such as eczema and pustular eruptions, conjunctivitis, pharyngitis, stomatitis, leucoplakia, gastritis and colitis. The skin may be sensitive to light. Inhalation of nickel or chromium compounds causes chronic nasal inflammation and bronchitis and in contact with the skin they may induce severe dermatitis. Phosphorus, arsenic, lead, manganese, copper, alcohol, chlorinated hydrocarbons, coal tar, etc. which predispose to tumour formation in the liver cause acute or chronic hepatitis before doing so. The bladder mucosa of aniline dye workers, who are predisposed to tumour formation at this site, exhibits inflammatory changes before the tumour appears. Smoking causes chronic inflammatory change of the bronchial mucosa with increased sensitivity to inhaled irritants. Chronic exposure of the skin to tar or purified carcinogenic agents leads to folliculitis, hyperaemia, inflammatory changes and hyperkeratoses and the skin may be sensitive to light. The agent responsible for the Shope skin tumour in rabbits likewise induces inflammatory changes and hyperkeratoses.

Following herpes zoster, which predisposes to benign and malignant tumour formation (Wyburn-Mason, 1955), the affected skin is over-sensitive to stimuli of all

* This concept is borne out by the recent observations on the effect of cortisone on tumour formation with chemical carcinogens (Duran-Reynals, personal communication). If mouse skin is painted with methylcholanthrene insufficiently to produce lesions, the injection of cortisone causes the appearance of both papillomata and carcinomata, suggesting that depression of macrophage function allows malignant cells to grow.

kinds and shows a tendency to become inflamed easily and for dermatitis and psoriasis to appear in the skin or leukoplakia in the mouth. Again the site of recurrent herpes zoster may be the seat of benign or malignant tumour formation (Wyburn-Mason 1957b). The affected tissues show an abnormal sensitivity to light or temperature changes. In the buccal mucosa malignant change is often preceded by chronic inflammatory leukoplakic changes which are also frequently found in the vagina prior to malignant change.

In all the above conditions the affected tissues exhibit inflammatory changes and increased sensitivity to noxious stimuli, indicative of disturbance of function in macrophages. The importance of stimuli in inducing malignant change is shown especially in the heredo-familial disease xeroderma pigmentosa, in which the normal protective secretions of the skin are deficient. Exposure of the skin to light, especially ultra-violet, leads to the development of hyperkeratosis, chronic inflammatory changes, papillomata and angiomata and there is a marked sensitivity to sunlight or other stimuli which may cause change to rodent ulcer, squamous carcinomata or malignant melanomata. In experimental tar cancers in animals injury of a carcinogenically prepared area may precipitate tumour formation (Deelman 1922, 1923, 1924; Pullinger 1940). Deelman and Van Erp (1926) showed that a crop of papillomata was precipitated along the line of deep incisions in mouse skin previously tarred. Berenblum and Shubik (1949) found that in mice brief treatment of the skin with a standard carcinogen would not produce a tumour. If, however, the areas were subsequently treated for a prolonged period with croton oil, itself non-carcinogenic, tumours arose in numbers approximately proportional to the concentration of the carcinogen used. Scarification, wounding, heating and freezing had a similar effect. The carcinogen acted as the "initiator" and the other agents as "promoters" of malignant invasion (Berenblum 1954). When brought into contact with skin urethane does not lead to inflammation but is an initiator of carcinogenesis. If a co-carcinogen is later applied malignant invasion occurs. *One essential step in carcinogenesis appears to be the causing of mutation by the carcinogen. This is instantaneous, irreversible and invisible. The so-called co-carcinogenic action of croton oil, injury and many other factors which act on macrophages is necessary to lead to malignant invasion* and determines its latent period (Berenblum). Berenblum considered the possibility that co-carcinogen worked by irritation, though why so many irritants are ineffective he could not explain.

(B) *Factors acting on Congenital Anomalous Tissues containing Mutant Cells.* It is well known that malignant change tends to be precipitated easily in congenital anomalies which have been shown to be composed of mutant cells. These include pigmented nævi, in which infection and trauma are recognized agents leading to malignant change, teratomata and ectopic testes, in which surgical intervention and trauma are also important factors leading to malignancy (Gordon-Taylor 1948). In cases of melanosis oculi the onset of malignancy is related to infection and trauma (Walsh 1947). In Von Recklinghausen's disease and other heredo-familial pre-cancerous diseases malignant change in the neurofibromata may take place especially after trauma or attempts at surgical removal (Wilson 1940).

Again there appear to be two factors of importance in causing malignant change

the existence of mutant cells surrounded by macrophages in the tissues and the occurrence of a stimulus leading to inflammatory changes and involving alteration in macrophage activity

(C) *Trauma Chronic Irritation and Inflammation preceding Malignant Change* As shown above non specific trauma chronic irritation and inflammation may precede benign and malignant tumour formation and the cell mutation occurs during the repair processes In such conditions there is of course an alteration of macrophage activity The tissues are abnormally sensitive to noxious stimuli

To summarize in almost all *premalignant conditions the tissues contain mutants and may show inflammatory changes and an exaggerated reaction to noxious stimuli of various kinds trauma light etc Malignant invasion is often precipitated by exposure to stimuli or agents producing inflammation including trauma These act on macrophages and either stimulate or kill them* Most carcinogenic agents not only cause cell mutation but also affect the phagocytic activity of macrophages and so alter the rate of cell division It has been shown that in certain circumstances malignant cells may exist in the body surrounded by macrophages and exhibit no tendency to invasion Similarly in benign tumours and congenital anomalies the individual mutant cells composing the lesions are surrounded by macrophages We have seen that agents which depress the phagocytic activity of macrophages appear to stimulate the rate of growth of malignant tumours The role of noxious agents in promoting malignant invasion may be that they stimulate macrophages and so cell division or they kill the macrophages lying around and between the mutant cells and so prevent the concentration of immune bodies in relation to the latter at the same time removing the macrophage mechanism by which the metabolic activity of cells is normally controlled Some of these mutant cells possess the ability to feed and divide without the intermediary of macrophages and emerge by a process of selective growth On the other hand it is possible that the change from a benign to a malignant tumour or the appearance of malignancy in a congenital anomaly might be the result of further mutation in the component cells of the lesion Apart from that seen in hormone dependent cancers spontaneous regression of tumours is occasionally observed and sometimes results from non specific causes such as infections operations etc which may also on the contrary stimulate tumour growth Such effects might well result from a non specific alteration of the activity of the macrophages around a tumour

Gliomata

In normal central nervous tissue the microglia is found everywhere surrounded and in relation to ganglion cells and axons the function of which they control Here also are found fine unmyelinated fibres No vital staining of unmyelinated nerve fibres and microglia is found within a glioma Macrophages form a barrier around the edge of these tumours In the more slowly growing tumours this may form a fibroblast like capsule The structure of gliomata is that of areas of dedifferentiated glial cells looking like astrocytes or oligodendrocytes medulloblasts or ependymal cells Often mixtures of all types of cell and also diffuse pleomorphism may be observed Gliomata are like malignant tumours of other tissues mutant cells growing without macrophage and neural control

Placental Tumours

Benign tumours such as fibromata or angiomata may occur in the placenta presumably as the result of somatic mutation in the appropriate cells. Malignant tumours or chorion epitheliomata may likewise develop in the placenta. Similar tissue may also be found in teratomata. In the former case they are probably the result of early failure or defective development of the embryo (see Willis). These malignant tumours give no staining with vital dyes and contain no macrophages or nerve fibres. They consist of chorionic cells probably arising by mutation during fœtal life. Their development in pregnancy is perhaps related to disturbed genetic and therefore immunological relationships between the fœtal material and that of the mother.

Congenital Tumours

The commonest congenital malignant tumours are the congenital sarcomata, the neuroblastomata, sympathicoblastomata and ganglioneuromata, Wilms tumours of the kidney and retinoblastomata (Wells 1940). All may be associated with congenital growth anomalies of the affected or other tissue. The tumours, like congenital benign tumours and abnormalities, presumably arise from cells which have undergone somatic mutation during early development.

Myeloid and Lymphatic Leukæmias

In leukæmias there is an excessive number of white cells and their immature forms in the blood and these are also found infiltrating all tissues where they continue to mature. In myeloid leukæmia granulopoiesis is disturbed, in lymphatic leukæmia the lymphoid tissue is primarily affected. It is necessary to separate from cases of true lymphatic leukæmia those cases of lymphoid reticuloses in which abnormal lymphoid cells appear in the circulation and which appear to be separate conditions. When considering the nature of the leukæmias and whether they are benign or malignant conditions, that is whether they metastasize, it is important to recall that blood cells and their precursors normally pass into the circulation and may settle down, proliferate and mature in any tissue.

Leukæmias may occasionally follow exposure to chemicals, especially benzol, pyridine and aniline dyes, indole, hydroxylamine, tar and benzanthracene, X rays, radium, etc., all of which may cause mutation in cells and benign and malignant tumour formation in various tissues. It seems reasonable to conclude that the basis of a leukæmia is a mutation in the precursor cells of a portion of the hæmopoietic system. This allows uncontrolled production of abnormal white cells which pass into the circulation and there mature. In any condition in which division of white cell precursors is stimulated, such as in infections or in polycythæmia, there is an increased chance of a mutation developing. Acute differ from chronic leukæmias not only in their course but in age incidence and response to therapeutic agents such as 6 mercapto purine. They are presumably due to different cell mutations.

Such abnormal white blood cells act as irritants and stimulate the reticulo endothelial cells of the various tissues in which they lie. Thus in lymph nodes vital staining shows a greatly increased number of reticulo endothelial cells as taking up

the dye This stimulation of reticulo endothelial cells must cause vasodilatation oedema local inflammatory changes and increases the uptake of circulating sub stances and metabolic processes and so growth of the cells of the infiltrated tissues leading to hyperplasia Thus the uptake of P^{3} is greatest in the tissues which show the greatest infiltration and irritation by leukæmic cells such as the liver spleen kidney and bone marrow (Warren 1943)

Because of these effects there are features common to both conditions which resemble those of an intoxication or infection These include skin lesions of various types such as generalized vasodilatation oedema inflammatory lesions such as mycosis fungoides eczema psoriasis and dermatitis arthritis etc and a rise in B M R and temperature all of which would seem to follow irritation of the macro phages of the tissues Hyperplastic changes found include hyperplasia of the liver salivary and lacrimal glands (Mickulicz s syndrome) stimulation of periosteal bone growth with osteoporosis hyperplasia of the gastro intestinal mucosa etc All these lesions are stuffed with abnormal white cells

Tumour Formation in Lower Forms

Reference has been made to the existence of elements with the character of reticulo endothelial cells in all vertebrates and in all invertebrates above and including the molluscs They are presumed to function in the same way as in vertebrates Fox (1923) Feldman (1932) Cramer (1932) Patchffe (1933) and Dobberstein (1953) found that nearly all neoplasms occurring in man also occur in vertebrate animals Cancer in animals appears to be due usually to chronic irritation It can be induced by X rays chemicals and hormone disturbances Pflugfelder (1953) found that growths also occur in urochordates molluscs annelids crustacea arthropods arachnids insects etc in which cells like macrophages are found Macrophages presumably perform the same function in relation to mutant malignant cells in lower animals as in man

APPENDIX CASE NOTES

Case 1

Male aged 58 years Family history—nil relevant Ten years before hospital admission he noticed gradual increase in the size of his hands and feet and alteration in his facial appearance A diagnosis of acromegaly was made Five years before admission he began to suffer from girdle pains around the trunk at the level of the lower part of the thorax weakness of the legs and impairment of sensation The physical signs indicated a lesion at the level of D8 segment Investigations showed no abnormality in the cerebro spinal fluid and intrathecal injection of lipiodol no block in the spinal subarachnoid space Laminectomy revealed the presence of an intramedullary tumour causing a fusiform enlargement in this region No treatment was possible The paralysis increased and two years before admission he developed bleeding piles and pain in the region of the anus particularly at night Considerable amounts of mucus were passed per rectum and occasional bouts of constipation occurred

Examination —The patient had a typical acromegalic appearance with prognathism and enormous hands and feet Multiple pedunculated pigmented moles and neurofibromata of the skin of small type were present over the trunk and limbs The signs of a central lesion of the cord were present as indicated by the severe spastic paraplegia extensor plantar responses and dissociated loss of pain and temperature sensibility extending from D5 above to L1 segment below Micturition was normal but there was no control over defæcation A papilliferous mass was present round the anus chiefly on the right side and an ulcer extended into the anal canal for about 1¼ inches The rectal lymph nodes were palpable on both sides The blood pressure was 165/100 mm Hg Biopsy from the rectal mass showed a dedifferentiated carcinoma of colloid type Cerebro spinal fluid was normal with a protein content of 35 mgm % blood count R B C 4 56 mill per cu mm Hb 88% C I 0 95 blood urea 32 mgm % fasting blood sugar 90 mgm W R and Kahn negative Radiographs of the skull showed disorganization of the walls of the pituitary fossa The clinoid processes were displaced relative to each other and the dorsum sellæ backwards and partially destroyed The floor of the fossa was not clear The appearances were those of a pituitary adenoma Well marked acromegalic changes were present in the facial bones Osteo arthritic changes were found in both hip joints The bones of the pelvis were osteoporotic and their trabecular structure coarse Gross osteo arthritic changes associated with alteration of the texture of the bones were present throughout the spinal column and in other bones and appeared to be due to an unusual form of Paget s disease The alkaline phosphatase content of the blood was 12 9 units (normal 5–10) and the acid phosphatase 4 5 units (normal 0 5–2 0)

Case 2

Female aged 35 years The patient was born with a left sided facial nævus Her heart was found to be abnormal at an early age At the age of 14 years a partial surgical excision of the nævus was carried out This left much scarring At the age of 17 the nævus had recurred and she was treated by the injection of boiling water into the nævus followed by surface application of 5 0 mgm of radium plaques At the age of 25 she was again admitted to hospital and the boiling water injections were repeated Since that time the changes had recurred and large veins had appeared over the left side of the face and temple The vision in the left eye had much deteriorated in the course of the years though that in the right was good *Examination* —The facial appearance of the patient is shown in Fig 71 Restricted to the whole of the left trigeminal area was a blue angiomatous condition with purple raised areas and much scarring The tissues were swollen and overgrown as compared with the normal The skin showed areas of hyperkeratosis and

soft warty growths and tended to hang down as a fold The right iris was blue and the left brown The left eyelids were much thickened and swollen The conjunctiva exhibited chemosis and was swollen The sclera was very red and took part in the angiomatous change Reddish purple vesicles were to be seen in it The globe of the left eye was markedly hypertrophic and generally larger than the right The left pinna was somewhat larger than the right Large varicose veins were present at the inner canthus and on the left temple The increase in size of the left face caused the nose to be noticeably pushed over to the right The teeth on the left side of the jaws were obviously larger than on the right side A loud systolic murmur could be heard over all areas of the chest especially in the mesocardial region The apex beat was not displaced The blood pressure was 110/70 mm Hg The other systems including the central nervous system were normal Radiographs of the skull showed an increase in size of all the left facial bones and teeth as compared with the right The flat bones on the left side showed a noteworthy rarefaction and appearances like those of Paget s disease the sutures being obliterated though those of the right side were visible The nasal septum was markedly over to the right side and the left clinoid processes and the left orbit were larger than the right Radiographs of the chest showed a prominence of the pulmonary arteries and right ventricle but no enlargement of the auricles or left ventricle The hilar markings were normal It seemed probable that a patent interventricular septum was present Biopsy of one of the warty growths showed it to be an angio lipo fibroma

CASE 3

Male aged 4 years Either at birth or within three weeks the face was observed to be asymmetrical It was thought at first to be due to a birth injury but gradually increased in severity At the age of 7 months he attended Great Ormond Street Hospital where he was found to have congenital left hemihypertrophy of the face with enlargement of the maxilla and left eye irregular and eccentric left pupil and right sided hydrocœle At the age of 2½ years iridectomy was performed on the left eye and later a small piece of bone was removed from the hard palate for microscopical examination This had an irregular lamellar structure with fibrosis consistent with some form of sclerosing osteitis such as Paget s disease or leontiasis ossea *Examination* —The only abnormality was in the left side of the face and head The left face was considerably larger than the right side and particularly in the lower part the mouth pulled over towards the right the left eyelid dropped The soft tissues of the cheek were thickened as were the gums and the left side of the hard palate The globe of the left eye was larger than the right There was an old iridectomy scar The thickening of the gums caused a partial burying of the teeth on the left side whereas those on the right were fully erupted X rays of the skull showed enlargement of the left sphenoidal fissure and orbit The lesser and greater wing of the sphenoid on the left side was greater than on the right in fact the whole of the left side of the anterior skull was larger than on the right The bones showed evidence of increase in density resembling that of Paget s disease The calcification of the teeth on the left side was considerably in advance of that on the right The incisor teeth on the left side sloped downwards and to the right and lower upwards and to the right This was in contrast to those on the right

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soft warty growths and tended to hang down as a fold The right iris was blue and the left brown The left eyelids were much thickened and swollen The conjunctiva exhibited chemosis and was swollen The sclera was very red and took part in the angiomatous change Reddish purple vesicles were to be seen in it The globe of the left eye was markedly hypertrophic and generally larger than the right The left pinna was somewhat larger than the right Large varicose veins were present at the inner canthus and on the left temple The increase in size of the left face caused the nose to be noticeably pushed over to the right The teeth on the left side of the jaws were obviously larger than on the right side A loud systolic murmur could be heard over all areas of the chest especially in the mesocardial region The apex beat was not displaced The blood pressure was 110/70 mm Hg The other systems including the central nervous system were normal Radiographs of the skull showed an increase in size of all the left facial bones and teeth as compared with the right The flat bones on the left side showed a noteworthy rarefaction and appearances like those of Paget s disease the sutures being obliterated though those of the right side were visible The nasal septum was markedly over to the right side and the left clinoid processes and the left orbit were larger than the right Radiographs of the chest showed a prominence of the pulmonary arteries and right ventricle but no enlarge ment of the auricles or left ventricle The hilar markings were normal It seemed probable that a patent interventricular septum was present Biopsy of one of the warty growths showed it to be an angio lipo fibroma

Case 3

Male aged 4 years Either at birth or within three weeks the face was observed to be asymmetrical It was thought at first to be due to a birth injury but gradually increased in severity At the age of 7 months he attended Great Ormond Street Hospital where he was found to have congenital left hemihypertrophy of the face with enlargement of the maxilla and left eye irregular and eccentric left pupil and right sided hydrocœle At the age of 2½ years iridectomy was performed on the left eye and later a small piece of bone was removed from the hard palate for microscopical examination This had an irregular lamellar structure with fibrosis consistent with some form of sclerosing osteitis such as Paget s disease or leontiasis ossea *Examination* —The only abnormality was in the left side of the face and head The left face was considerably larger than the right side and particularly in the lower part the mouth pulled over towards the right the left eyelid dropped The soft tissues of the cheek were thickened as were the gums and the left side of the hard palate The globe of the left eye was larger than the right There was an old iridectomy scar The thickening of the gums caused a partial burying of the teeth on the left side whereas those on the right were fully erupted X rays of the skull showed enlargement of the left sphenoidal fissure and orbit The lesser and greater wing of the sphenoid on the left side was greater than on the right in fact the whole of the left side of the anterior skull was larger than on the right The bones showed evidence of increase in density resembling that of Paget s disease The calcification of the teeth on the left side was considerably in advance of that on the right The incisor teeth on the left side sloped downwards and to the right and lower upwards and to the right This was in contrast to those on the right

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